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Aspects on Extracorporeal Life Support for severe acute respiratory failure with special reference to the influenza A/H1N1 2009 pandemic

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Karolinska Institutet

Stockholm 2016

Published by Karolinska Institutet.

ISBN 978-91-7676-255-4
Printed by Eprint AB 2016

Aspects on Extracorporeal Life Support for severe acute respiratory failure with special reference to the influenza A/H1N1 2009 pandemic

THESIS FOR DOCTORAL DEGREE (Ph.D.)

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"The rule is that hypoxia must be treated first, because hypoxia kills quickly while hypercapnia kills slowly."

JF Nunn, *Nunn's Applied Respiratory Physiology*

To my children

ABSTRACT

Extracorporeal membrane oxygenation provides pulmonary and circulatory support. First abandoned from the majority of the intensive care community because of disappointing results in several trials (with questionable methods), it experienced a renaissance during the H1N1 influenza pandemic in 2009/2010. Today, it is a widely accepted therapy option for the sickest respiratory failure patients, with survival rates between 50% and 80%, depending on the mode and the center where it is performed. But there is still a lack of high quality randomized trials to answer the question whether ECMO is superior to conventional mechanical ventilation in severe respiratory failure. But such trials are very difficult to perform mainly due to ethical reasons because a randomization to death is not acceptable for the majority of health care professionals. The question we have to ask ourselves today is not whether ECMO should be performed but how it should be performed, and here there is still a lot of work to do.

In this thesis, several aspects on extracorporeal life support for severe respiratory failure were investigated.

Paper I describes the treatment strategies and short-term outcome of 13 patients with refractory severe respiratory failure due to infection with influenza A H1N1 2009 at the ECMO Department at the Karolinska University Hospital. All patients survived, and 12 were still alive 3 months after discharge from ECMO.

In **paper II** the ECMOnet score is presented. It was developed by the Italian ECMOnet, and the 13 patients treated due to H1N1 infection in paper I were included in an external validation group for this score. It has a high accuracy for the prediction of mortality risk in the patients treated with veno-venous ECMO for H1N1 respiratory failure. The probability of correctly classifying patients with this score was 75%, where a score of 4.5 was the most appropriate cutoff for prediction of mortality risk. In the external validation group, the score had a good capacity to distinguish survivors from non-survivors.

Paper III is a neurocognitive long-term follow-up study of seven of the patients presented in paper I. The studied showed that despite prolonged episodes of hypoxemia, cognitive functioning was normal in all patients and that there were no hypoxic cerebral lesions.

Severe respiratory failure is a hyperinflammatory condition, and neutrophil granulocytes play a key role in its development and progress. In **paper IV**, we explored the hypothesis that the change in proportions of mature and immature neutrophils could be used as a prognostic parameter during the course of ECMO treatment, but due to a low number of included patients, no strong conclusion could be drawn.

The systematic review presented in **paper V** is a result of question that arose in paper III, namely whether hypoxemia during the course of acute respiratory failure or ECMO treatment per se is associated with short- and long-term cognitive dysfunction in survivors. There are no high quality studies addressing this question, and it is therefore still not clear whether there is a causal relationship between hypoxemia and cognitive impairment. New studies are needed to investigate this important question because it is evident that different treatment strategies of acute respiratory failure have an impact on survival.

LIST OF SCIENTIFIC PAPERS

This doctoral thesis is based on the following five papers.

Holzgraefe B, Broomé M, Kalzén H, Konrad D, Palmér K, Frenckner B. Extracorporeal membrane oxygenation for pandemic H1N1 2009 respiratory failure. Minerva Anestesiol. 2010;76(12):1043-51

F. Pappalardo, M. Pieri, T. Greco, N. Patroniti, A. Pesenti, A. Arcadipane, VM Ranieri, L Gattinoni, **B Holzgraefe**, G Beutel, A Zangrillo on behalf of the Italian ECMOnet. Predicting mortality risk in patients undergoing venovenous ECMO for ARDS due to Influenza A (H1N1) pneumonia: the ECMOnet score.
Intensive Care Med 2013;39(2):275-81

B. Holzgraefe, C. Andersson, H. Kalzén, V. v. Bahr, M. Mosskin, E-M. Larsson, K. Palmér, B. Frenckner, A. Larsson. Does permissive hypoxemia during Extracoporeal Membrane Oxygenation causes long-term neurological impairment? A study in patients with H1N1 induced respiratory failure.
Eur J Anaesthesiol 2016; 33:DOI:10.1097/EJA.0000000000000544

B. Holzgraefe, P. Jones, B. Frenckner, S. Eksborg, O. Winqvist, A. Larsson. Neutrophil heterogeneity during Extracorporeal Membrane Oxygenation for severe respiratory failure: a prospective, observational pilot study.
In manuscript 2016

B. Holzgraefe, L. von Kobyletzki, A. Larsson. The effect of hypoxemia on cognitive outcome in patients with severe acute respiratory failure treated with conventional mechanical ventilation or Extracorporeal Membrane Oxygenation – a systematic review of the literature. In manuscript 2016

CONTENTS

List of abbreviations

ALL	Acute lymphatic leukemia
ARDS	Acute respiratory distress syndrome
BIPAP	Bilevel positive airway pressure
BMI	Body mass index
BSA	Body surface area
CaO_2	Arterial oxygen content
CD	Cluster of differentiation
$CD16^{hi}$	Mature neutrophil granulocyte
$CD16^{int}$	Immature neutrophil granulocyte
CDH	Congenital diaphragmatic hernia
CI	Confidence interval
COPD	Chronic obstructive pulmonary disease
CPAP	Continuous positive airway pressure
CRP	C- reactive protein
CRRT	Continuous renal replacement therapy
CVP	Central venous pressure
CXCR	Chemokine receptor
ECMO	Extracorporeal membrane oxygenation
ECPR	Extracorporeal cardiopulmonary resuscitation
ECLS	Extracorporeal life support
ELSO	Extracorporeal Life Support Organization
FACS	Flourescence-activated cell sorting
FITC	Fluorescein isothiocyanate
FSIQ	Full scale intelligence quotient
GAI	General ability index
G-CSF	Granulocyte colony-stimulating factor
GM-CSF	Granulocyte-macrophage colony-stimulating factor
GRO	Growth-regulated oncogene
Hb	Hemoglobin
HC	Healthy control

ICU	Intensive care unit
IFN-a	Interferon alfa
IFN-g	Interferon gamma
IL	Interleukin
IL-1Ra	Interleukin-1receptor antagonist
IL-12p40	Interleukin-12 p40 subunit
IMV	Invasive mechanical ventilation
IP-10	Interferon gamma inducible protein 10
IQR	Interquartile range
MAS	Meconium aspiration syndrome
MCP	Monocyte chemoattractant protein
MDC	Macrophage-derived chemokine
MI	Memory function idex
MIP	Macrophage inflammatory protein
MV	Minute ventilation
NIV	Non-invasive ventilation
OI	Oxygenation index
$PaCO_2$	Arterial carbon dioxide tension
PaO_2	Arterial oxygen tension
PCP	Pneumocystis jiroveci pneumonia
PCD	Programmed cell death
PEEP	Positive endexpiratory pressure
P/F ratio	Ratio of arterial oxygen tension and inspiratory oxygen fraction
PMN	Polymorphonuclear neutrophil
PPHN/PFC	Persistent pulmonary hypertension/persistent foramen ovale
PRISMA	Preferred reporting items for systematic reviews and meta-analysis
PE	Phycoerythrin
PCT	Procalcitonin
PIP	Peak inspiratory pressure
Paw	Mean airway pressure
PBW	Predicted body weight

PVL	Panton valentine leukocidin
RCT	Randomized controlled trial
ROC	Receiver operating characteristic
SaO_2	Arterial oxygen saturation
SD	Standard deviation
SIMV	Synchronized intermittent mandatory ventilation
SOFA	Sequential organ failure assessment score
TNF-a	Tumor necrosis factor alpha
TNF-b	Tumor necrosis factor beta
Va+v	Veno-arterial plus venous
v-a ECMO	Veno-arterial ECMO
VA-VV	Veno-arterial to veno-venous
VEGF	Vascular endothelial growth factor
VILI	Ventilator induced lung injury
V_T	Tidal volume
VVA	Veno-veno-arterial
v-v ECMO	Veno-venous ECMO
VVDL	Veno-venous double lumen
VVDL+V	Veno-venous double lumen + venous
VV-VA	Veno-venous to veno-arterial
WBC	White blood cell count
WHO	World Health Organization

1 INTRODUCTION

Severe respiratory failure and the acute respiratory distress syndrome

In his classic textbook of applied respiratory physiology, the author, JF Nunn, describes severe respiratory failure as "a pathophysiological condition that negatively affects the ability of a patient to maintain normal arterial oxygen or carbon dioxide partial pressures, which is of pulmonary but not cardiac origin" [1].

This definition agrees with what used in the work by Asbaugh and co-workers, who described a new form of respiratory failure in 12 patients admitted to their intensive care unit (ICU) with refractory hypoxemia despite substitution of oxygen with (n = 7) or without (n = 3) mechanical ventilation. In two patients, the measurements of partial pressure of oxygen and carbon dioxide were performed with room air. Table 1 shows the respiratory data in this article. The table was reprinted with kindly permission of *the Lancet*, where the work was published in 1967.

Table 1. Respiratory characteristics of the 12 reported cases in the paper by Asbaugh and colleagues in *the Lancet,* 1967

TABLE II—RESPIRATORY DATA

Case	Respiratory support	Frequency (min.$^{-1}$)	$\dot{V}_E$(l./min.)	S_aO_2	$P_{IO_2}-P_{aO_2}$ gradient (mm. Hg)	P_aCO_2 (mm. Hg)	pH	Compliance (l./cm. water)
1	Nasal oxygen (8 l./min.)	40	16·0	85	..	45·0	7·360	0·016
2	Bennett respirator (P.R.I.) (100% oxygen) ..	28	16·8	72	..	62·0	7·245	0·016
3	Bennett respirator (P.R.I.) (100% oxygen) ..	48	..	78	..	40·0	7·410	..
4	Bennett respirator (P.R.I.) (100% oxygen) ..	48	..	73	..	47·0	7·330	..
5	Engstrom respirator (70% oxygen) ..	48	8·0	85	..	63·0	7·270	..
6	Room air	36	14·4	84·4	..	37·0	7·338	0·017
7	Oxygen mask (3 l./min.)	64	..	79	..	22·0	7·420	..
8	Room air	44	20·0	41	536 (100% oxygen)	29·5	7·395	0·009
9	Bennett respirator (vol.) (80% oxygen) ..	20	8·0	84	320 (100% oxygen)	57·5	7·270	0·016
10	Nasal oxygen (7 l./min.)	48	48·0	87	..	30·5	7·420	0·019
11	Bennett respirator (P.R.I.) (40% oxygen) ..	36	25·2	74	..	29·5	7·480	0·017
12	Bennett respirator (P.R.I.) (40% oxygen) ..	34	12·8	72	220 (100% oxygen)	30·0	7·450	0·013

$\dot{V}_E$ = Expired volume. S_aO_2 = Arterial oxygen saturation.
P_aCO_2 = Partial pressure of carbon dioxide in arterial blood.
$P_{IO_2}-P_{aO_2}$ gradient = Oxygen-tension gradient between inspired gas and arterial blood.
P.R.I. and vol. indicate the model of Bennett respirator.

Table reprinted with permission of The Lancet.[2]

The clinical situation was featured by bilateral infiltrates in the chest X-ray of all patients as well as low compliance of the respiratory system. Seven of 12 patients died. The authors called it acute respiratory distress syndrome (ARDS) as it occurred independently from the cause of respiratory failure and without cardiac failure [2].

Today the term ARDS is well established in the speciality of intensive care medicine and describes a condition of respiratory failure without referring to a specific underlying cause. In a recent a publication, ARDS is defined as an inflammatory disorder in the lungs and classified as mild, moderate, or severe depending on the severity of hypoxemia, appearance of infiltrates on the chest radiogram, and positive end-expiratory pressure (PEEP) settings during mechanical ventilation [3].

The four criteria which have to be fulfilled for acute respiratory failure to be termed ARDS according the recent definition ("the Berlin definition of ARDS") are listed below [3, 4].

1. Timing: the onset of respiratory failure may not be older than 1 week.
2. No lung edema caused by cardiac failure or fluid overload.
3. Bilateral radiological opacities in the chest X-ray (in severe ARDS the opacities have to be at least in three quadrants).
4. Hypoxemia
   - mild ARDS: P/F ratio 201–300 mmHg with a peep ≥ 5 cm H_20
   - moderate ARDS: P/F ratio ≤ 200 mmHg, peep ≥ 5 cm H_20
   - severe ARDS: P/F ratio ≤ 100 mmHg, peep ≥ 10 cm H_20

All studies in this thesis report findings in patients who had developed severe ARDS according to above described Berlin definition. Therefore the term severe ARDS will be used in the following.

Pathophysiology

According to a classification proposed by Gattinoni et al. I distinguish between pulmonary and extrapulmonary ARDS. When the lungs are directly affected from the epithelial side, e.g., in bacterial or viral pneumonia, it is called pulmonary ARDS, whereas extra-pulmonary ARDS develops from the endothelial side because of primary infectious or inflammatory diseases outside of the lungs, i.e., intraabdominal sepsis [5]. However, as already stated above, the etiology of severe respiratory failure and ARDS may be different, but the clinical features, which are hypoxemia, infiltrates in the chest X-ray, decreased compliance of the respiratory system, and need of advanced mechanical ventilation are similar but of different severity depending on the category of ARDS. The histological appearance and mechanisms on the cellular level are also very similar despite different underlying causes. The histological picture can be divided into an acute and a chronic stage, as described in "Nunn's applied respiratory physiology" [1]. During the acute stage, epithelial cells are damaged, which causes intraalveolar and interstitial edema with concomitant gas exchange disturbances. In the later phase, called the chronic or fibroproliterative stage, damaged or destroyed tissues are repaired. In this stage, thickening of the layers (epithelium, endothelium, interstitium) and fibroproliferative rebuilding occur. Interstitial edema is still present, and pulmonary function is improving, but still seriously compromised. In the most severely ill patients considerable lung-parenchymal and lung function abnormalities are still present 1 year after treatment [1, 6, 7].

ARDS is an inflammatory condition, and neutrophil granulocytes (polymorphonuclear neutrophil = PMN), which are the first line defense cells in acute infection and/or inflammation, seem to play an important role in development and maintaining of this syndrome [8,9]. PMNs are attracted by specific chemokines, mainly interleukin 8 (IL-8) [8]. The PMNs have surface receptors, CXCR1 and CXCR2, which have a high affinity for IL-8. The CXCR2 receptor is downregulated in sepsis and septic ARDS and therefore PMNs expressing CXCR1 are considered the most important [10]. These PMNs migrate from peripheral blood to the pulmonary capillary bed and from there into the lung parenchyma where their activation continues by mediators that are released from other

inflammatory cells (macrophages, lymphocytes, endothelial cells) but also from other kinds of neutrophils [1,8]. Experimental and clinical studies suggest that PMNs not only act as defenders against the inflammatory insult, they also contribute to the development and maintaining of ARDS itself [8,9,11].

The major reasons for the impact of PMNs on the development and progress of ARDS are, as we understand them today, an imbalance between the defense against pathogens, with inappropriately high release of pro-inflammatory substances (e.g., cytokines, chemokines, and free radicals) attracting PMNs to the lungs, and a decreased rate of apoptosis, i.e., programmed cell death (PCD), clearing PMNs from the lungs [12].

Epidemiology of ARDS

In a recently published observational study, the authors collected patient data from 459 ICUs worldwide during a four-week period in the winter of 2014. There were 2377 patients who developed ARDS from a total of 29,144 patients admitted to an ICU. Citation from [13]: "The period prevalence of mild ARDS was 30.0% (95% CI: 21.7%–25.2%); of moderate ARDS, 46.6% (95% CI: 44.5%–48.6%); and of severe ARDS, 23.4% (95% CI: 21.7%–25.2%). The hospital mortality was 46.1% (95% CI: 41.9%–50.4%) for patients with severe ARDS and the overall mortality 40%" [13]. These numbers are similar to the findings from other studies where the overall mortality rates ranged from 34% to 43% [14-16].

Treatment

As stated above, common features of ARDS are hypoxemia with or without hypercapnia. Depending on their severity, these patients are treated with either supplemental oxygen during spontaneous breathing, non-invasive (NIV), or invasive mechanical ventilation (IMV). High ventilatory driving pressures (i.e. the difference between end-inspiratory and end-expiratory airway pressures), high end-inspiratory airway pressures, and high tidal volumes during conventional mechanical ventilation could further damage the lungs, leading to ventilator-induced lung injury (VILI) [17–19].

There is some evidence that certain treatment strategies can reduce short- or long-term mortality in patients suffering from ARDS: a) mechanical ventilation with lower tidal volumes (V_T), b) prone positioning, and c) referral to an institution with the possibility to perform extracorporeal membrane oxygenation (ECMO) [20-23]. However, a) low V_T ventilation has not been studied for patients with severe ARDS, b) prone positioning showed only a mortality advantage in patients with severe ARDS, and c) ECMO treatment has not been investigated for the mild or moderate forms of ARDS.

Despite the fact that mortality from ARDS has decreased over time, it remains still unacceptably high, about 40% [14].

EXTRACORPOREAL MEMBRANE OXYGENATION

ECMO provides partial or total extracorporeal organ support in case of the most severe forms of respiratory, cardiac, or combined cardiorespiratory failure. ECMO is a very invasive, technically advanced, and expensive treatment, which requires a certain caseload of at least 30 treatments per year to achieve higher survival rates compared to low volume centers [24]. Therefore, in my view, only experienced centers should provide ECMO. These centers also need a dedicated transportation organization as most patients have to be referred, and a referral on ECMO seems to be safer compared to conventional transportation in this category of patients [25].

Indications and contraindications for respiratory ECMO

Indications for ECMO are severe refractory hypoxemic or hypoxic respiratory failure with or without circulatory failure. In the guidelines from the Extracorporeal Life Support Organization (ELSO), it is recommended to consider ECMO when the estimated mortality without ECMO is ≥ 50%, and ECMO is indicated when the mortality risk is > 80%. Thus, according to this guideline ECMO is indicated when the partial pressure of oxygen in the arterial blood is < 100 mmg (7.5 kPa) with an inspired oxygen concentration of > 90% (P/F ratio < 100 mmHg) [26]. In a recent trial, patients were randomized to referral to an ECMO center or conventional treatment with transfer when the "Murray" lung injury score > 3. Murray and co-workers developed this score in 1988 to better classify the severity of lung injury [27]. It takes into account the lung mechanics during mechanical ventilation, the degree of hypoxemia, and the occurrence of opacities in the chest X-ray (Table 2)

Table 2. The lung injury score developed by Murray et al.
Table reprinted with permission from the American Journal of Respiratory and Critical Care Medicine

COMPONENTS AND INDIVIDUAL VALUES OF THE LUNG INJURY SCORE*

		Value
1. Chest roentgenogram score		
No alveolar consolidation		0
Alveolar consolidation confined to 1 quadrant		1
Alveolar consolidation confined to 2 quadrants		2
Alveolar consolidation confined to 3 quadrants		3
Alveolar consolidation in all 4 quadrants		4
2. Hypoxemia score		
Pao_2/FIo_2	≥300	0
Pao_2/FIo_2	225–299	1
Pao_2/FIo_2	175–224	2
Pao_2/FIo_2	100–174	3
Pao_2/FIo_2	<100	4
3. PEEP score (when ventilated)		
PEEP	≥5 cm H_2O	0
PEEP	6–8 cm H_2O	1
PEEP	9–11 cm H_2O	2
PEEP	12–14 cm H_2O	3
PEEP	≥15 cm H_2O	4
4. Respiratory system compliance score (when available)		
Compliance	≥80 ml/cm H_2O	0
Compliance	60–79 ml/cm H_2O	1
Compliance	40–59 ml/cm H_2O	2
Compliance	20–39 ml/cm H_2O	3
Compliance	≤19 ml/cm H_2O	4

The final value is obtained by dividing the aggregate sum by the number of components that were used

	Score
No lung injury	0
Mild-to-moderate lung injury	0.1–2.5
Severe lung injury (ARDS)	>2.5

However, as most patients treated with ECMO do not die because of hypoxemia but due to secondary complications, e.g., intracranial bleeding or septic multi-organ failure, oxygenation scores are only helpful in the decision when to start ECMO but not in predicting the possible outcome of this treatment.

Other indications for ECMO are refractory hypercapnia despite maximal conventional ventilation with a blood pH < 7.2, severe barotrauma with air leakage, or bridge to lung transplantation.
Contraindications for ECMO are terminal malignancy, known non-recoverable diseases, and massive intracranial hemorrhage.
Other situations, e.g., prolonged mechanical ventilation or immunosuppression, are not any longer considered as contraindications and should be evaluated individually.

Technique

We distinguish between veno-venous and veno-arterial ECMO (v-v ECMO and v-a ECMO). V-v ECMO is used for pulmonary support in severe respiratory failure with maintained cardiac function and v-a ECMO in patients with severe refractory cardiac or cardiorespiratory failure. The patients' blood is always drained from the venous site of circulation by a centrifugal or roller pump (Figures 1 and 2).

Figure 1. ECMO apparatus with centrifugal pump

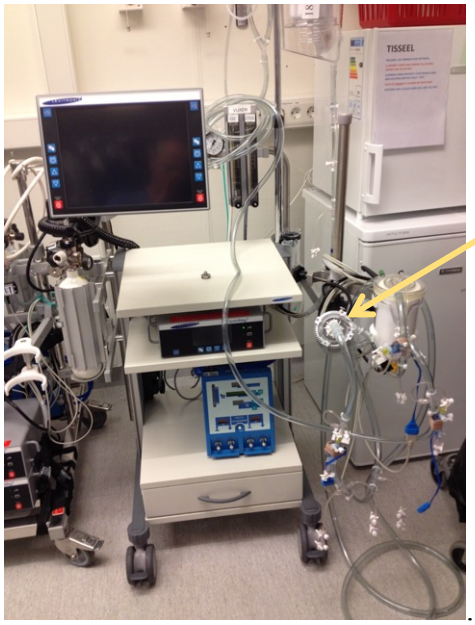
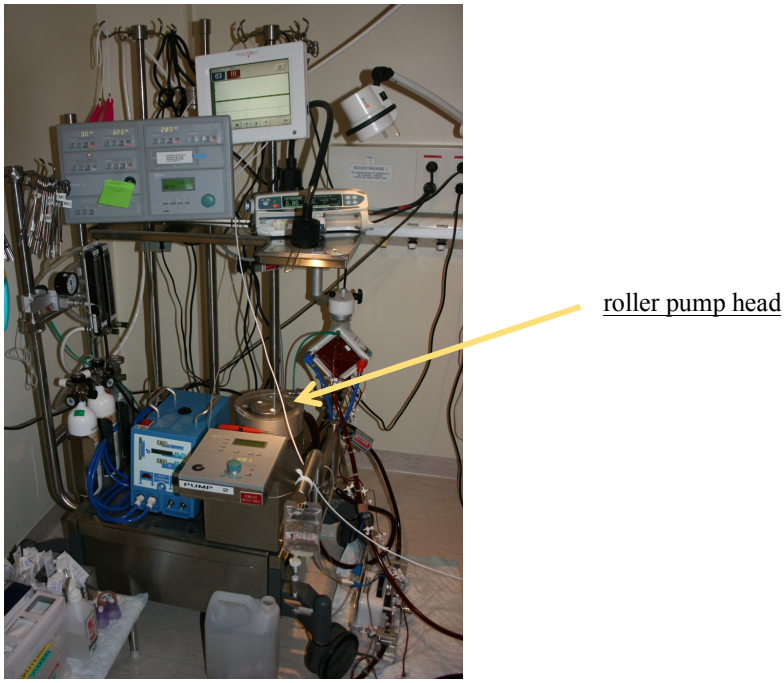
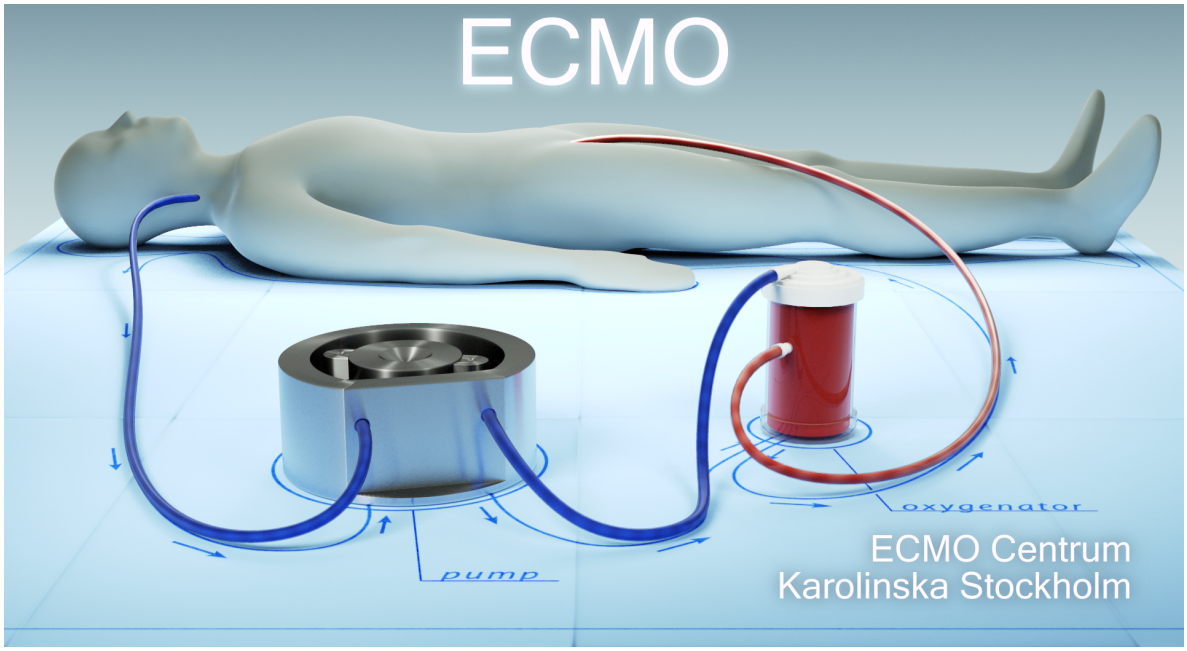


Figure 2. ECMO apparatus with a roller pump head



The desaturated blood then passes through the artificial lung (the oxygenator) where the gas exchange takes place and then it returns either into a vein or an artery depending on the indication for ECMO (Figure 3).

Figure 3. v-v and v-a ECMO, flow direction.



Courtesy of Anders Lidén, Lidén Film 2009, Stockholm, Sweden

Today, there are two general methods to cannulate the vessels of a patient for v-v ECMO, either with a double lumen cannula, which is usually inserted via the right internal jugular vein, or with a two-site cannulation technique where the draining cannula is also inserted via the right internal jugular vein and the returning cannula into the right or left femoral vein. The two-site technique is

the preferred cannulation method at the ECMO Department Karolinska. Most centers prefer to drain desaturated venous blood via the inferior vena cava and return the saturated blood through the superior vena cava into the right atrium. With this approach, it is possible to achieve higher saturations due to reduced blood recirculation compared to the technique at the ECMO Department Karolinska [28,29]. However, from my clinical experiences, the limitation of the former approach is that the total extracorporeal flow, i.e., the level of oxygen delivery by ECMO that can be reached, is less with this method than with the method used at the ECMO Department Karolinska, particularly in the awake and dehydrated patient, as the venous drainage through the IVC is dependent on the hydration of the patient and the intraabdominal pressure. In v-a ECMO venous drainage from the superior vena cava instead of from inferior vena cava seems to be advantageous with respect to the saturation of the upper body, which has been shown in an experimental study [30].

ECMO Department Karolinska

The ECMO Department of the Karolinska University Hospital is a six-bed ECMO ICU that is dedicated to ECMO treatment of neonatal, pediatric, and adult patients with severe respiratory and circulatory failure. Since 1987, more than 1000 patients have been treated here with ECMO. The ECMO Center has also performed more than 700 ECMO transports since 1996 when the transportation organization was established [31].

Outcome of ECMO treatment

In 1989, the Extracorporeal Life Support Organization (ELSO) was founded in the USA. The intention was to form an organization for ECMO providing centers and to develop this treatment. In 2015, a total of 367 centers were members of the ELSO and data from over 69,000 ECMO treatments have been collected in a worldwide database. In the following tables (3–10) from the ELSO database, the outcome of ECMO treatment are displayed as international and center-specific summaries [32].

Table 3. International summary of ECMO treatments: number and category of patients and outcome (1989–2015)

ECLS Registry Report
International Summary
July, 2015

Extracorporeal Life Support Organization
2800 Plymouth Road
Building 300, Room 303
Ann Arbor, MI 48109

Overall Outcomes

	Total Patients	*Survived ECLS*		*Survived to DC or Transfer*	
Neonatal					
Respiratory	28,271	23,791	84%	20,978	74%
Cardiac	6,046	3,750	62%	2,497	41%
ECPR	1,188	766	64%	489	41%
Pediatric					
Respiratory	6,929	4,579	66%	3,979	57%
Cardiac	7,668	5,084	66%	3,878	51%
ECPR	2,583	1,432	55%	1,070	41%
Adult					
Respiratory	7,922	5,209	66%	4,576	58%
Cardiac	6,522	3,661	56%	2,708	42%
ECPR	1,985	791	40%	589	30%
Total	69,114	49,063	71%	40,764	59%

Table 4. Center-specific ECMO treatments at the ECMO Department Karolinska: number and category of patients and outcome (1989–2015)

ECLS Registry Report
Center Specific Summary
July, 2015
ECMO Centrum Karolinska (36)

Extracorporeal Life Support Organization
2800 Plymouth Road
Building 300, Room 303
Ann Arbor, MI 48109

Overall Outcomes

	Total Patients	*Survived ECLS*		*Survived to DC or Transfer*	
Neonatal					
Respiratory	323	275	85%	272	84%
Cardiac	14	8	57%	8	57%
ECPR	6	2	33%	2	33%
Pediatric					
Respiratory	170	129	76%	128	75%
Cardiac	11	8	73%	8	73%
ECPR	44	12	27%	11	25%
Adult					
Respiratory	297	200	67%	198	67%
Cardiac	6	5	83%	5	83%
ECPR	43	12	28%	12	28%
Total	914	651	71%	644	70%

Table 5. Respiratory treatments in neonatal patients in the international summary: diagnoses and ECMO modes.

International Summary - July, 2015

Neonatal Respiratory Runs by Diagnosis

	Total Runs	*Avg Run Time*	*Longest Run Time*	*Survived*	*% Survived*
CDH	7,419	256	2549	3,793	51%
MAS	8,815	133	1327	8,253	94%
PPHN/PFC	4,915	155	1225	3,790	77%
RDS	1,553	136	1093	1,305	84%
Sepsis	2,873	143	1200	2,093	73%
Pneumonia	381	248	1002	222	58%
Air Leak Syndrome	133	171	979	98	74%
Other	2,591	185	1843	1,573	61%

Run time in hours. Survived = survival to discharge or transfer based on number of runs

Neonatal Respiratory Support Mode Details

	Total Runs	*Avg Run Time*	*Longest Run Time*	*Survived*	*% Survived*
VA	19,176	182	2549	13,581	71%
VVDL	5,828	149	1429	4,917	84%
VA+V	1,412	182	1176	1,024	73%
VV-VA	830	244	1229	515	62%
VVDL+V	745	157	682	593	80%
VV	554	165	1227	412	74%
Unknown	69	177	1072	41	59%
VA-VV	36	277	956	24	67%
Other	28	235	956	19	68%
VVA	2	98	158	1	50%

Run time in hours. Survived = survival to discharge or transfer based on number of runs

Table 6. Number of ECMO treatments for respiratory failure in neonatal patients at the ECMO Department Karolinska: diagnoses and ECMO modes.

ECMO Centrum Karolinska (36) Center Specific Summary - July, 2015

Neonatal Respiratory Runs by Diagnosis

	Total Runs	*Avg Run Time*	*Longest Run Time*	*Survived*	*% Survived*
CDH	93	304	885	70	75%
MAS	125	100	525	124	99%
PPHN/PFC	37	240	1090	23	62%
RDS	13	164	710	12	92%
Sepsis	27	147	476	22	81%
Pneumonia	8	386	914	5	63%
Air Leak Syndrome	3	85	129	3	100%
Other	33	231	956	20	61%

Run time in hours. Survived = survival to discharge or transfer based on number of runs

Neonatal Respiratory Support Mode Details

	Total Runs	*Avg Run Time*	*Longest Run Time*	*Survived*	*% Survived*
VA	188	224	1090	145	77%
VVDL	106	109	639	101	95%
VV	21	112	250	21	100%
VV-VA	11	388	914	4	36%
VA+V	6	446	796	3	50%
VA-VV	5	505	885	4	80%
Unknown	1			1	100%
Other	1	956	956	0	0%

Run time in hours. Survived = survival to discharge or transfer based on number of runs

Table 7. Number of ECMO treatments for respiratory failure in pediatric patients in the international summary: diagnoses and ECMO modes.

International Summary - July, 2015

Pediatric Respiratory Runs by Diagnosis

	Total Runs	*Avg Run Time*	*Longest Run Time*	*Survived*	*% Survived*
Viral pneumonia	1,530	317	2968	997	65%
Bacterial pneumonia	712	286	1411	420	59%
Pneumocystis pneumonia	35	373	1144	18	51%
Aspiration pneumonia	311	246	2437	213	68%
ARDS, postop/trauma	187	247	935	117	63%
ARDS, not postop/trauma	560	305	3086	302	54%
Acute resp failure, non-ARDS	1,261	257	2718	689	55%
Other	2,477	219	2465	1,286	52%

Run time in hours. Survived = survival to discharge or transfer based on number of runs

Pediatric Respiratory Support Mode Details

	Total Runs	*Avg Run Time*	*Longest Run Time*	*Survived*	*% Survived*
VA	3,466	253	2781	1,797	52%
VVDL	1,344	250	3086	938	70%
VV	1,141	259	2437	727	64%
VV-VA	411	363	1715	189	46%
VA+V	283	237	1483	128	45%
VVDL+V	228	284	1198	145	64%
Other	77	392	2968	49	64%
Unknown	57	276	1932	32	56%
VA-VV	54	454	1466	33	61%
VVA	12	357	985	4	33%

Run time in hours. Survived = survival to discharge or transfer based on number of runs

Table 8. Number of ECMO treatments for respiratory failure in pediatric patients at the ECMO Department Karolinska: diagnoses and ECMO modes

ECMO Centrum Karolinska (36) Center Specific Summary - July, 2015

Pediatric Respiratory Runs by Diagnosis

	Total Runs	*Avg Run Time*	*Longest Run Time*	*Survived*	*% Survived*
Viral pneumonia	32	298	1193	21	66%
Bacterial pneumonia	34	318	1231	27	79%
Pneumocystis pneumonia	7	470	1144	6	86%
Aspiration pneumonia	19	184	1044	19	100%
ARDS, postop/trauma	7	239	871	6	86%
ARDS, not postop/trauma	11	365	1481	6	55%
Acute resp failure, non-ARDS	10	308	1103	5	50%
Other	61	203	1720	42	69%

Run time in hours. Survived = survival to discharge or transfer based on number of runs

Pediatric Respiratory Support Mode Details

	Total Runs	*Avg Run Time*	*Longest Run Time*	*Survived*	*% Survived*
VV	68	245	1231	54	79%
VA	66	276	1720	41	62%
VV-VA	16	439	1103	14	88%
VVDL	15	107	325	10	67%
VA-VV	8	337	626	8	100%
Other	3	529	814	2	67%
VVDL+V	2	163	211	2	100%
VA+V	2	27	36	0	0%
VVA	1	136	136	1	100%

Run time in hours. Survived = survival to discharge or transfer based on number of runs

Table 9. Number of ECMO treatments for respiratory failure in adult patients in the international summary: diagnoses and ECMO modes

International Summary - July, 2015

Adult Respiratory Runs by Diagnosis

	Total Runs	*Avg Run Time*	*Longest Run Time*	*Survived*	*% Survived*
Viral pneumonia	688	327	3208	459	67%
Bacterial pneumonia	1,118	255	3288	687	61%
Aspiration pneumonia	168	235	2634	106	63%
ARDS, postop/trauma	408	254	1993	230	56%
ARDS, not postop/trauma	733	313	6248	398	54%
Acute resp failure, non-ARDS	1,378	266	4527	761	55%
Other	3,637	235	6745	2,024	56%

Run time in hours. Survived = survival to discharge or transfer based on number of runs

Adult Respiratory Support Mode Details

	Total Runs	*Avg Run Time*	*Longest Run Time*	*Survived*	*% Survived*
VV	4,140	256	5014	2,451	59%
VVDL	2,002	273	3208	1,306	65%
VA	900	184	6745	401	45%
Not Collected	498	232	1525	254	51%
VV-VA	202	428	3018	59	29%
VVDL+V	118	386	4527	77	65%
VA-VV	109	328	1521	57	52%
VA+V	59	154	808	24	41%
Other	57	391	6248	27	47%
VVA	45	351	1993	9	20%

Run time in hours. Survived = survival to discharge or transfer based on number of runs

Table 10. Number of ECMO treatments for respiratory failure in adult patients at the ECMO Department Karolinska: diagnoses and ECMO modes

ECMO Centrum Karolinska (36) Center Specific Summary - July, 2015

Adult Respiratory Runs by Diagnosis

	Total Runs	*Avg Run Time*	*Longest Run Time*	*Survived*	*% Survived*
Viral pneumonia	8	342	1117	7	88%
Bacterial pneumonia	95	273	1585	60	63%
Aspiration pneumonia	13	217	646	10	77%
ARDS, postop/trauma	19	315	1315	14	74%
ARDS, not postop/trauma	32	322	2205	18	56%
Acute resp failure, non-ARDS	11	328	1012	7	64%
Other	123	386	6745	84	68%

Run time in hours. Survived = survival to discharge or transfer based on number of runs

Adult Respiratory Support Mode Details

	Total Runs	*Avg Run Time*	*Longest Run Time*	*Survived*	*% Survived*
VV	146	205	1417	117	80%
VA	71	361	6745	48	68%
VV-VA	60	605	1585	19	32%
VVDL	8	211	646	5	63%
VA-VV	7	504	681	6	86%
Not Collected	6	234	305	3	50%
VA+V	3	53	90	2	67%

Run time in hours. Survived = survival to discharge or transfer based on number of runs

Influenza A/H1N1 2009

In April 2009, the World Health Organization (WHO) defined the outbreak of a novel viral infection, the swine origin influenza A H1N1 2009 as an international health problem. The first infections were diagnosed in Mexico during March 2009, and in June 2009 the WHO declared it a pandemic [33].

In most patients the infection was mild, but in the patients who developed hypoxemic respiratory failure and who were in need of ventilatory support in an ICU, the clinical situation quickly deteriorated further in many cases, necessitating ECMO treatment. Between 2009 and January 2016, a total of 53 patients were treated with ECMO at our unit due to confirmed infection with influenza A or B. In 45 of these patients, influenza A could be diagnosed and of these 37 had influenza A H1N1. It seems that patients with pneumonitis due to infection with H1N1 more often develop severe refractory hypoxemic respiratory failure with a need for ECMO compared to other types of Influenza A or B. However, to the best of my knowledge, this hypothesis has not been scientifically investigated.

Hypoxemia and Hypoxia

The occurrence of hypoxemia is a common feature in severe ARDS and other conditions with severely impaired pulmonary gas exchange. It is defined as a hemoglobin oxygen saturation below the normal value. The term "normoxemia" is defined as a PaO_2 of 10.7–13.3 kPa (80–100 mmHg) or a saturation of > 94% at sea level [34,35]. On the other hand, the term hypoxia describes a lack of oxygen at the cellular level. Hypoxemia and hypoxia are often mixed up, but it is important to keep in mind that they describe two totally different situations. It is possible to survive a hypoxemic condition without hypoxia, but it is not possible to survive a long-lasting hypoxic condition because "hypoxia kills quickly" [1]. Hypoxemia may not lead to cellular hypoxia per se because the physiological compensation of reductions in oxygen saturation will keep capillary oxygen content up [36]. Tissue hypoxia is usually caused by an ischemic event, i.e., cerebral infarction. Studies of oxygenation in healthy participants on high altitude expeditions have shown that it is possible to tolerate hypoxemia quite well [37]. However, there seems to be a risk for cerebral lesions and microhemorrhages in association with high altitude, i.e., hypobaric hypoxemia [38]. Nevertheless, these conditions are difficult to compare with those seen in patients with an underlying disease suffering from hypoxemic respiratory failure. However, in these patients, it could be possible to accept hypoxemia as long as the circulation is preserved. Unfortunately, it is difficult to define a lower oxygenation threshold that is still acceptable and safe, especially if tissue oxygenation is not taken into account [39].

2 AIMS

The aims of the presented studies in this dissertation are

I. To describe the characteristics, clinical features, the treatment, and the short-term outcome of 13 patients who were suffering from severe hypoxemic respiratory failure after infection

with the pandemic influenza A H1N1 2009 virus and treated with ECMO at the ECMO Department Karolinska.

II. To identify possible predictors of mortality in patients suffering from H1N1 hyoxemic respiratory failure who are treated with ECMO.

III. To investigate the possible effect of permissive hypoxemia on the prevalence of cognitive impairment and brain lesions in a long-term follow-up study in survivors from study I.

IV. To study the hypothesis regarding whether changes in circulating neutrophil subsets during ECMO treatment for severe refractory respiratory failure have a diagnostic and/or prognostic value.

V. To systematically review the current literature about the possible relationship between hypoxemia and cognitive dysfunction in patients treated with mechanical ventilation or ECMO for severe respiratory failure.

3 MATERIALS AND METHODS

Ethics

The local ethical committee of Stockholm approved the studies I–IV. Studies I and II were covered by the same ethical approval: DNR-2009/1849-31/2; study III: DNR-2012/986-31/3 and study IV: DNR-2011/1282-31/1. Informed consent was obtained by the patients or their relatives when the patient was unable to give consent, and in children the consent was obtained by the parents. Paper V is a systematic literature review, and for this kind of work no ethical approval is needed.

Patients, study methods, and statistics

I. This study was an observational investigation of all patients with confirmed pandemic influenza A H1N1 2009 severe ARDS treated with ECMO at the ECMO Department Karolinska. Patient data for evaluation were routinely collected during the treatment and retrospectively analyzed after the approval of the local ethics committee and after obtaining informed consent by the patients or theire nearest relatives when patients were not able to give consent. The treatment modalities, clinical routines at the ECMO Department, and the short-term outcome are described in this paper. The data analysis in this study was descriptive, and medians with interquartile ranges are presented; no other statistical methods were used.

II. The study for the second paper was performed in Italy by the Italian ECMOnet, which was established during the H1N1 pandemic. Data from 60 patients with confirmed or suspected influenza A H1N1 infection from 14 ICUs in Italy which had been prospectively collected during the pandemic by the Italian ECMOnet were analyzed [40]. With these data a multivariable analysis was performed to develop a model to predict morality in patients eligible for ECMO treatment because of severe respiratory failure. Variables statistically associated with mortality ($p \leq 0.25$) were included into a univariate analysis. From this, the data with a $p = 0.01$ were further analyzed by multivariate analysis to develop the ECMOnet score. The data from study I were part of an external validation group to confirm the results of the Italian patient cohort.

III. Long-term survivors of the ECMO-treated patient cohort during the pandemic 2009 were invited to participate in a follow-up study 3 years after ECMO treatment. To evaluate their cognitive function, a standardized cognitive test battery was applied. These tests were performed by a senior psychologist, experienced in testing cognitive functioning. To estimate the arterial oxygen content (CaO2) during ECMO the PaO_2 was calculated according to to the formula published by Severinghaus: $Ln\ PaO_2 = 0.385\ ln\ (S^{-1} - 1)^{-1} + 3.32 - (72\ S)^{-1} - 0.17\ (S^6)$, where S is the peripherally measured saturation [41]. Possible hypoxic brain lesions were studied by magnetic resonance imaging. The examinations were independently assessed by two senior neuroradiologists. The data are displayed as means with 95% confidence intervals (CI) where appropriate.

IV. The proportions of mature ($CD16^{hi}$) and immature ($CD16^{int}$) neutrophil granulocytes of the total number of CD16 positive cells, serum concentrations of cytokines, chemokines, and routinely used inflammatory parameters (c-reactive protein, procalcitonin, and white blood cell count) were studied in ECMO patients. Cells in heparinized whole blood from six patients, three children and three adults, treated with ECMO for severe ARDS and sepsis and one healthy control (HC) were stained with monoclonal antibodies and analyzed by fluorescence-activated cell sorting (FACS). Blood samples were analyzed at two different time points, after admission to the ECMO department and before decannulation. The time points were named ECMO early and ECMO late. The serum concentrations of cytokines and chemokines (Eotaxin, G-CSF, GM-CSF, Fraktalkine, IFN-α, IFN-γ, GRO, IL-10, MCP-3, IL-12p40, MDC, IL-15, IL-1Ra, sIL2 Ra, IL-1a, IL-4, IL-5, IL-6, IL-7, IL-8, IP-10, MCP-1, MIP-1β, TNF-α, TNF-β, VEGF) were analyzed by Luminex at the Department of Clinical Immunology of the Karolinska University Hospital. C- reactive protein, procalcitonin and white blood cell count (CRP, PCT, WBC) were analyzed daily during the entire treatment by the Department of Clinical Chemistry, Karolinska University Hospital. The values at the beginning and the end of the ECMO treatment in our unit are displayed. The hypothesis was tested with a paired *t*-test if the Shapiro-Wilk normality test was passed. Changes in the other variables were tested with paired *t*-test or with the Wilcoxon Signed Rank test if the normality test failed. The mean with 1 SD of the proportions of $CD16^{hi}$ and $CD16^{int}$ and their CXCR1 expression and of serum concentrations of cytokines, chemokines, CRP, PCT and WBCs are displayed for the two different time points: ECMO early and ECMO late. When the normality test failed, the median with interquartile range (IQR) is displayed. The difference between ECMO early and late was considered significant, with a two-tailed *p* value of <0.05.

V. It is known that patients with a chronically hypoxemic situation, e.g., chronic obstructive pulmonary disease (COPD), can develop cognitive sequelae [42, 43]. As described in paper III, the ECMO Center Karolinska accepts lower hemoglobin oxygen saturations during ECMO than usually recommended. After the results of the long-term follow-up study in paper III, we conducted a systematic review of the current literature to study whether there is proof for the hypothesis that hypoxemia in patients with acute severe respiratory failure is responsible for the development of short- and long-term cognitive impairment.

4 RESULTS

I

Between July 20, 2009, and January 12, 2010, 13 patients with H1N1 related severe ARDS were treated with ECMO at the ECMO Department of the Karolinska University Hospital (**Table 11**). Median age was 31 years (25–50). Five patients were previous healthy. Five patients presented with co-morbidities and had a median body mass index (BMI, kg/m^2) of 35 (31–42). A person with a BMI of 30 or more is generally considered obese according to the world health organization's (WHO) definition [44].

Table 11 showing patients' characteristics before cannulation for and before decannulation from ECMO

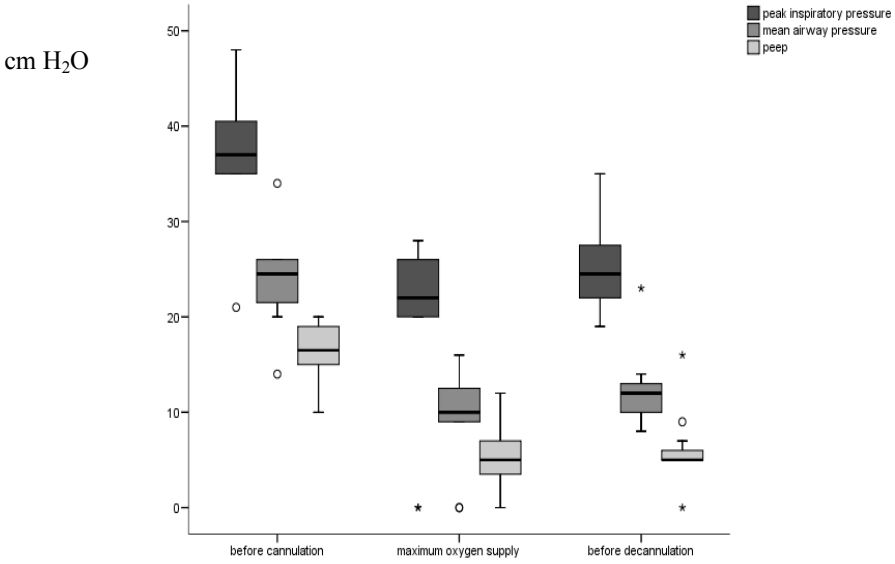
Parameter	Pre - ECMO	Prior to decannulation
N	13	13
Age (years)	31 (25-50)	
Female (pregnant)	5 (3)	5 (2)
Weight (kg)	88.5 (71-110)	75 (66-105)
FiO_2	1	0.6 (0.46-0.63)
P/F ratio	52.5 (38-60)	136 (99-226)
Murray score	3.6 (3.3-4)	-
Vent before ECMO (days)	1 (0.5-7)	-
Pip (cm H_2O)	37 (31-38)	25 (22-29)
Paw (cm H_2O)	25 (21-26)	12 (10-13)
Peep (cm H_2O)	17 (15-20)	5 (5-8)
Tv (ml)	545 (408-617)	577 (381-675)
Mv (L/min)	12.2 (9.1-15.2)	12.3 (9.8-17.3)
PaO_2 (kPa)	7 (5.1-7.8)	10.7 (8.7-13.1)
$PaCO_2$ (kPa)	6.3 (5.5-7.6)	5.6 (4.8-6.1)
SaO_2 (%)	86 (70-98)	95 (94-99)

P/F ratio: ration of arterial oxygen tension and inspiratory oxygen fraction, Pip: peak inspiratory pressure, Paw: mean airway pressure, Peep: positive end-expiratory pressure, Tv: tidal volume, Mv: minute volume, PaO_2: arterial oxygen tension, $PaCO_2$: arterial carbon dioxide tension, SaO_2: arterial oxygen saturation.

Median tidal volume in the 13 patients before ECMO was 7.2 mL/kg body weight (IQR 5.4–8.1 mL/kg). Median number of ventilator days before ECMO was 1 day (IQR 0.57 days). During ECMO treatment, as a lung rest strategy, ventilatory pressures and tidal volumes were effectively reduced (Figures 4 and 5).

Twelve patients were cannulated for v-v ECMO at the referring hospital by the ECMO Center's transportation team and then transported to our unit by ambulance (n = 7) or fixed wing aircraft/ambulance (n = 5). Two patients were transferred from foreign countries, one from Ireland and one from Scotland. One patient was cannulated for v-a ECMO at the Karolinska University hospital because of a combination of refractory cardiorespiratory failure.

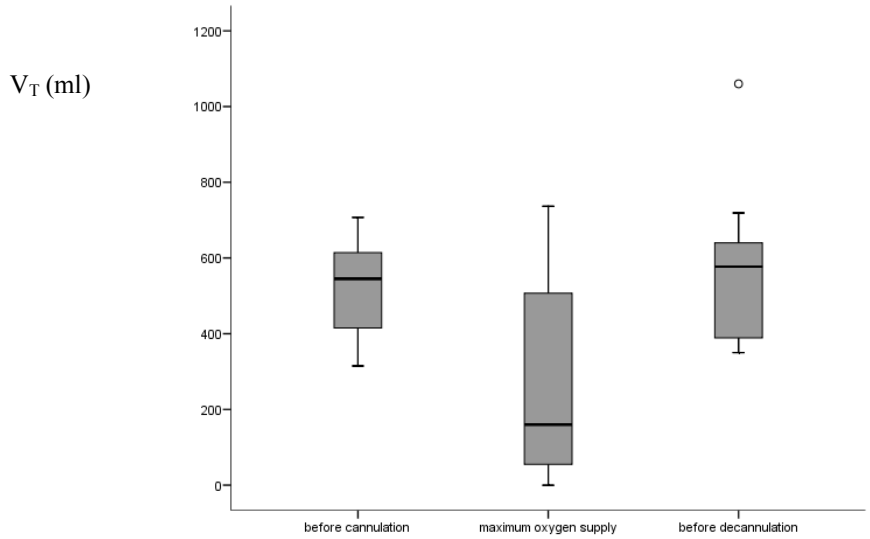
Figure 4. Airway pressures before and during ECMO treatment in 13 patients with severe ARDS due to infection with pandemic influenza virus A/H1N1 2009



Ventilatory pressures

Boxplots show the median, interquartile range, outliers, and extreme cases of individual cases. Data from one patient before cannulation were missing.

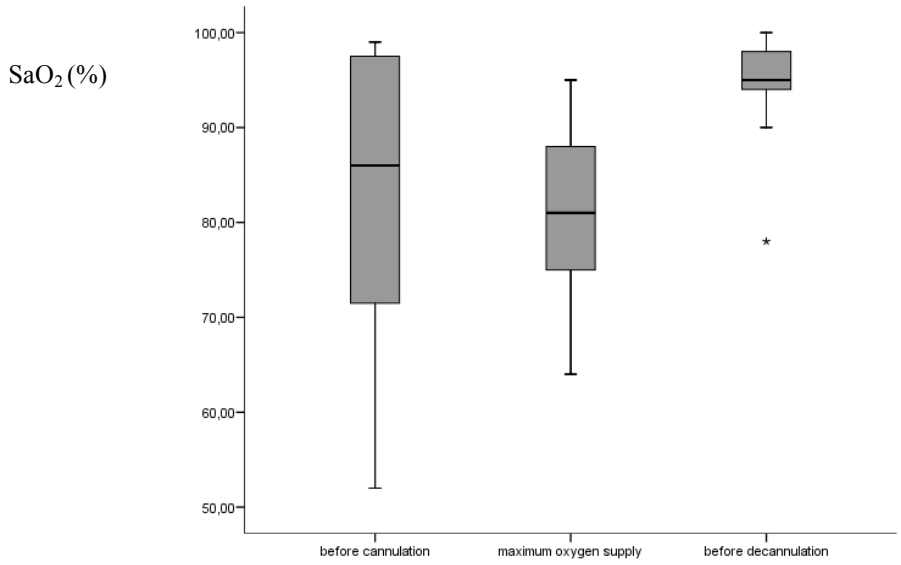
Figure 5. Tidal volume (V_T) before and during ECMO in 13 patients with severe ARDS due to infection with pandemic influenza virus A/H1N1 2009



Boxplots show the median, interquartile range, outliers, and extreme cases of individual cases. Data from one patient before cannulation were missing.

A drawback of this strategy is that it often leads to a loss of tidal volumes and end-expiratory lung volume, and sometimes it even leads to totally atelectatic lungs, with concomitant decrease of the hemoglobin oxygen saturation (**Figure 6**).

Figure 6. Boxplot of peripheral arterial saturation



Boxplots show the median, interquartile range, outliers, and extreme cases of individual cases. Data from one patient before cannulation were missing.

Complications

In eight patients, ECMO circuit complications occurred that required interventions: 20 changes of the entire ECMO circuit due to coagulation disorders (15 changes) or hemolysis (5 changes); three acute changes of the centrifugal pump due to formation of clots resulting in pump stops, two changes of the oxygenator because of malfunction, one procedure with evacuation of thrombi in the venous cannula, and one change of the raceway in a roller pump due to clot formation. None of these complications harmed the patient.

In one patient, an accidental liver puncture occurred during an attempt to puncture the right pleura for fluid drainage. The subsequent hemorrhage could be stopped 72 hours later after several laparotomies. Three patients developed pneumothorax and mediastinal emphysema during ECMO. Cannulation of the pleura with a large-bore chest drain for the evacuation of air may result in significant bleeding according to our experience. Therefore we chose to discontinue mechanical ventilation until the air had resolved (Figure 7).

Figure 7. A patient on v-a ECMO without mechanical ventilation because of right-sided pneumothorax.

The median duration of ECMO treatment was 16 days (IQR 9.5–30.5 days). All patients survived ECMO, one patient died 4 days after ECMO due to intracranial hemorrhage. Twelve patients survived for 3 months after discharge from the ECMO unit.

II

Forty-nine of 60 patients included in Italy had confirmed infection with the influenza A/H1N1 2009 virus. The survival rate was 71% in these patients. The remaining 11 patients without H1N1 infection had a survival rate of 54%.

In the univariate analysis, 20 variables were statistically associated with mortality, with a p value ≤ 0.25 (Table 12). Five predictors of death were then identified by using multivariate analysis: preECMO hospital length of stay (OR = 1.52, 95 % CI 1.12-2.07, $p = 0.008$), bilirubin value (OR = 2.32. 95% CI: 1.52–3.52, $p < 0.001$), systemic mean arterial pressure (OR = 0.92, 95% CI: 0.88–0.97, $p < 0.001$, hematocrit value (OR = 0.82, 95% CI: 0.72–0.94, $p = 0.008$) and creatinine level (OR = 7.38, 95% CI: 1.43–38.11, $p = 0.02$; Table 13). These five parameters were then entered into the score. This score was a statistically significant predictor of mortality (OR = 3.44, 95% CI: 2.04–5.81, $p < 0.001$). An ECMOnet score of 4.5 was found to have a probability of correctly classifying patients of 75%.

Table 12. Patient characteristics before ECMO implementation: comparisons between survivors and non-survivors

Variable	Total (*n* = 60)	Alive (*n* = 41)	Dead (*n* = 19)	P
Age, years	*40 ± 12.0*	*38 ± 12.9*	*43 ± 9.0*	*0.02*
Gender (male)	36 (60%)	26 (63%)	10 (53%)	0.6
Height, cm	169 ± 14.7	169± 17.3	168± 6.3	0.8
BMI, kg/m2	30.5 ± 8.8	30.7 ± 9.6	30.2 ± 6.9	0.8
BSA, m2	2.01 ± 0.38	2.03 ± 0.43	1.99 ± 0.25	0.6
PBW, kg	62 ± 11.8	63± 13.3	61± 7.4	0.5
Weight, Kg	88 ± 28.8	90 ± 32.2	86 ± 20.3	0.5
H1N1 confirmed	*49 (81%)*	*35 (85%)*	*14 (74%)*	*0.1*
PreECMO hospital stay, days	*2 (1-5)*	*2 (1-4)*	*6 (1-12)*	*0.02*
Hospital stay, days	*39 (24-50)*	*43 (29-53)*	*25 (16-45)*	*0.03*
ICU stay, days	20.5 (14-35)	22 (14-34)	19 (11-43)	0.4
MV, hours	18.5 (13-35.5)	19 (14-33)	18 (12-44)	0.8
Transport on ELS	*28 (47%)*	*21 (51%)*	*7 (37%)*	*0.2*
COPD	7 (12%)	4 (9.8%)	3 (15.8)	0.5
Heart disease	1 (1.7%)	1 (2.4%)	0	0.9
Smoke	*8 (13.3)*	*7 (17.1)*	*1 (5.3)*	*0.2*
Diabetes	*5 (8.3%)*	*5 (12%)*	*0*	*0.2*
Pregnancy	4 (6.7%)	4 (9.8)	0	0.3
Neoplasia	1 (1.7%)	1 (2.4%)	0	0.9
Psychopathology or alcoholic patient	6 (10%)	5 (12%)	1 (5.3%)	0.4
Rescue therapy:	42 (70%)	29 (71%)	13 (68%)	0.8
-Recruitment maneuvers	41 (68%)	28 (68%)	13 (68%)	0.9
-Nitric Oxide	10 (17%)	7 (17%)	3 (16%)	0.9
-Pronation	16 (28%)	12 (30%)	4 (21%)	0.6
- Pulmonary vasodilator	*5 (8.5%)*	*2 (4.9)*	*3 (16.7)*	*0.2*
Vasoactive amines	*20 (33%)*	*7 (17%)*	*13 (68%)*	*<0.001*
BIPAP	9 (15%)	7 (17%)	2 (11%)	0.3
HFOV	4 (6.8%)	2 (4.9%)	2 (11%)	0.4
Vasoactive and inotropic drugs	37 (65%)	25 (66%)	12 (63%)	0.5
CPAP-PSV	3 (5%)	3 (7.3%)	0	0.5
SIMV	2 (3.3%)	2 (4.8%)	0	0.9
SOFA score	*7.8 ± 2.2*	*7.0 ± 2.2*	*9.3 ± 3.2*	*<0.001*
Bilirubin, mg/dl	*0.90 (0.61-1.51)*	*0.80 (0.60-1.10)*	*1.18 (0.67-1.84)*	*0.02*
Cardiac Index, l/min/m²	*3.7 ± 1.6*	*3.9 ± 1.9*	*3.3 ± 1.1*	*0.1*
Cardiac Output, l/min	*7.3 ± 3.1*	*7.7 ± 3.2*	*6.6 ± 2.8*	*0.2*
Creatinine, mg/dl	*0.82 (0.65-1.21)*	*0.80 (0.60-1.08)*	*0.86 (0.67-1.90)*	*<0.001*
Hematocrit, %	*33 ± 5.6*	*35± 4.7*	*30 ± 6.8*	*0.01*
Heart rate, bpm	104 ± 21.0	102.8 ± 21.7	106.6 ± 19.7	0.3
Respiratory rate, bpm	27 ± 8.1	26 ± 8.7	29 ± 6.4	0.3
Lactate, mmol/l	*1.95 (1.20-2.55)*	*1.85 (1.20-2.40)*	*2.07 (1.20-3.00)*	*0.2*
PaCO2, mmHg	63 ± 20.1	64 ± 22.4	61 ± 14.2	0.6
MAP, mmHg	*77± 15.7*	*79 ± 16.4*	*71± 12.3*	*0,007*
PCV, mmHg	38 (63%)	25 (61%)	13 (68%)	0.6
PEEP, cmH2O	16 ± 3.8	16 ± 3.2	15 ± 4.9	0.4
pH	7.3 ± 0.1	7.3 ± 0.1	7.3 ± 0.1	0.5
Platelet count, x 103/µl	192 ± 125.2	190 ± 92.8	194± 175.1	0.9
Peak airway pressure, cmH2O	*35 ± 6.8*	*34 ± 5.9*	*37 ± 8.3*	*0.2*
Plateau airway pressure, cmH2O	*33 ± 4.6*	*34 ± 5.7*	*3 ± 2.2*	*0.1*
Mean airway pressure, cmH2O	25 ± 3.4	25 ± 3.4	26 ± 3.3	0.5
CVP, mmHg	*14 ± 4.7*	*15 ± 4.0*	*13 ± 5.6*	*0.2*
Volume-controlled mechanical ventilation	23 (38%)	15 (37%)	8 (42.1)	0.7
Vt, ml	405 ± 159.4	417 ± 171.7	379 ± 127.4	0.3
Minute volume, L	9.8 ± 4.5	9.9 ± 4.4	9.8 ± 4.6	0.9
Femoral vein-femoral vein configuration	*26 (43.3)*	*20 (48.8)*	*6 (31.6)*	*0.2*
Femoral vein-jugular vein configuration	27 (45)	18 (43.9)	9 (47.4)	0.8
Internal jugular vein-jugular vein configuration	6 (10)	3 (7.3)	3 (15.8)	0.3
Cannulation-related complications	*9 (15)*	*4 (9.8)*	*5 (26.3)*	*0.05*

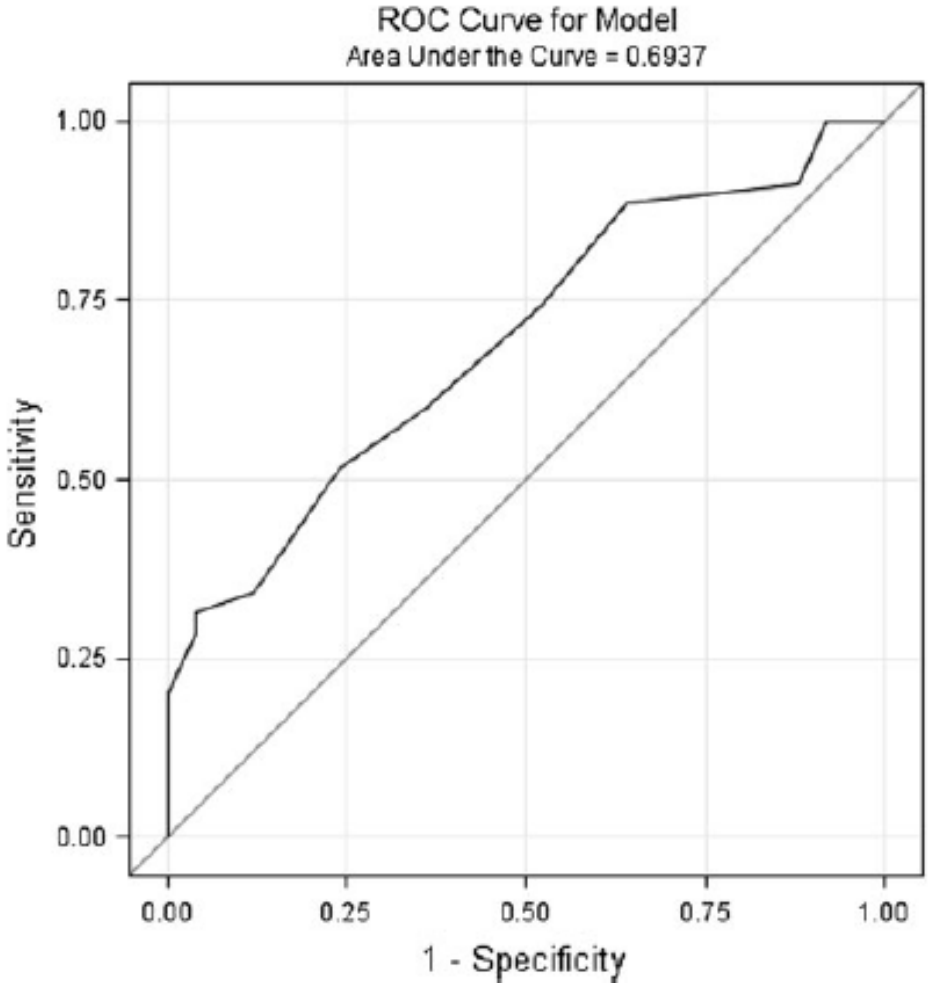
BMI: body mass index; BSA: body surface area; PBW: partial weight bearing; ICU: intensive care unit; MV: mechanical ventilation; ELS: extracorporeal life support; COPD: chronic obstructive pulmonary disease; BIPAP: bilevel positive airway pressure; HFOV: high-frequency oscillatory ventilation; CPAP PSV: continuous positive airway pressure and pressure support ventilation; CRRT: continuous renal replacement therapy; SIMV: invasive mechanical ventilation synchronized; SOFA: sequential organ failure assessment; $PaCO_2$: partial pressure of carbon dioxide; MAP: mean arterial pressure; PCV: pressure control ventilation; PEEP: positive end expiratory pressure; CVP: central venous pressure; Vt: tidal volume.

Table 13. The ECMOnet score

Parameter	Partial score
PreECMO hospital length of stay (days)	
≤3	0.5
4–7	1
8–11	1.5
>11	2
Bilirubin (mg/dl)	
≤0.15	0
0.16–0.65	0.5
0.66–1.15	1
1.16–1.65	1.5
1.66–2.15	2
>2.15	2.5
Creatinine (mg/dl)	
≤0.5	0
0.51–0.80	0.5
0.81–1.10	1
1.11–1.40	1.5
1.41–1.70	2
1.71–2.00	2.5
2.01–2.30	3
>2.30	3.5
Hematocrit (%)	
>40	0.5
36–40	1
31–35	1.5
≤30	2.0
Mean arterial pressure (mmHg)	
>90	0
61–90	0.5
≤60	1

The ROC analysis of the external validation group showed a strong capacity of the ECMOnet score to distinguish survivors from non-survivors (c = 0.694, 95 % CI: 0.562–0.862, p = 0.004). The accuracy was 62% and sensitivity and specificity were 51% and 76%, respectively (Figure 8)

Figure 8. ROC curve of the ECMOnet score in the external validation group.



III

13 patients survived ECMO and eleven were still alive 3 years after ECMO. Three out of eleven survivors were living abroad and eight in Sweden. We were able to contact one of the three patients living abroad and six patients in Sweden. They agreed to participate in this study (*n* = 7), the mean age was 31 years.

All patients were hypoxemic and five patients had also respiratory or metabolic acidosis at initiation of the ECMO treatment (Table 14).

Table 14. Ventilatory and oxygenation parameters before ECMO.

Patient #	Sex	Age (years)	Peep (mbar)	Pip (mbar)	Mean awp (mbar)	P/F ratio (mmHg)	OI	Murray score	pCO_2 (kPa)	pH
1	m	26	20	37	missing	54	missing	4	8.0	7,3
2	f	24	15	37	23	36	64	4	6.5	7.2
3	f	31	20	37	25	51	43	4	4.6	7.3
4	m	57	15	43	24	36	67	3.5	6.4	7.4
5	m	25	15	34	20	54	37	4	9.4	7.2
6	m	46	18	35	26	45	58	4	5.9	7.3
7	m	34	16	30	missing	60	missing	missing	missing	missing

Peep: positive end-expiratory pressure; Pip: peak inspiratory pressure; Mean awp: mean airway pressure; FiO_2: fraction of inspired Oxygen; P/F ratio: ratio of oxygen partial pressure in arterial blood to inspired oxygen fraction, FiO_2 = 1.0; OI: oxygenation index. In patient #1 the mean airway pressure was not registered before cannulation and in patient #7 the pre ECMO data are missing as ECMO was started in another department.

All patients had low saturations compared to traditional goals in severe ARDS but sufficient oxygen content and distribution during ECMO treatment (Table 15). Mean CaO_2 in the study population during the study period was 14.3 ± 1.9 ml/100 ml (mean ± SD) mean Hb 126 ± 8.5 g/L, mean venous pH 7.37 ± 0.03 and mean body temperature 37.2 ± 0.4°C during the observation period. $SpaO_2$ in all seven patients was registered 1191 times during the first 10 days.

Table 15. Mean values (±SD) of oxygenation parameters, tissue perfusion surrogates and blood transfusions during the observation period.

Patient #	$SpaO_2$ %	Hb (g l^{-1})	CaO_2 (ml dl^{-1})	SvO_2 %	Lactate (mmol l^{-1})	PRBC (ml)	ECMO days
1	72 ±8	131 ±13	13.2 ±1.7	73 ± 4	1.2 ±0.2	691 ±910	49
2	74 ±9	122 ±6	12.7 ±1.7	74 ± 5	2.2 ±1.2	262 ±204	23
3	83 ±5	117 ±7	13.8 ±1,3	76 ± 3	1.2 ±0.6	411 ±611	37
4	91 ±4	133 ±4	16.6 ±0.7	82 ± 5	1.5 ±0.3	251 ±435	3
5	75 ±7	120 ±7	12.6 ±1.3	74 ± 5	1.1 ±0.3	447 ±374	16
6	90 ±4	142 ±8	17.8 ±1.3	73 ± 6	1.5 ±0.3	58 ±143	7
7	79 ±11	119 ±6	13.2 ±2	69 ± 5	1.1 ±0.3	624 ±651	44
Group mean ±SD	**80 ±7.1**	**126 ±8.5**	**14.3 ±1.9**	**74 ± 3.7**	**1.4 ± 0.4**	**392 ± 205**	

SvO_2: preoxygenator venous saturation in the ECMO system; $SpaO_2$: peripheral measured hemoglobin Oxygen saturation; CaO_2: arterial oxygen content; ECMO days: duration of ECMO from cannulation to decannulation. PRBC: packed red blood cells; ml transfused during the first ten days of ECMO treatment or entire treatment if shorter than ten days. Group mean refers to the mean value of the individual patients mean values and SD to the standard deviation between individual patients mean values.

Global cognitive functioning (FSIQ and GAI) was at or above the average of 50% of the reference population (FSIQ 85-115) in five study patients and in one patient below this average (Table 16). Memory functioning (MI) was normal in all seven study patients when compared to age-matched healthy controls. Patient #2 could not be tested with FSIQ due to the lack of formal education, which is necessary for FSIQ (Table 16). The four index scores that are the components of FSIQ are also displayed.

Table 16. Cognitive and memory function, mean values (95% CI).

Patient #	Education (years)	FSIQ	GAI	MI	VCI	PRI	WMI	PSI
1	12	102(96-108)	104 (97-110)	107 (100-115)	108 (100-115)	98 (91-105)	105 (97-112)	95 (86-105)
2	<5	missing	missing	108 (100-115)	missing	missing	missing	missing
3	12	79 (74-85)	83 (77-90)	87 (82-96)	89 (82-97)	82 (76-90)	76 (70-85)	76 (70-89)
4	9	88 (83-94)	93 (87-100)	86 (79-93)	91 (84-99)	98 (91-105)	79 (73-88)	90 (82-101)
5	20	107 (101-112)	115 (108-121)	125 (116-130)	108 (100-115)	118 (110-124)	122 (113-128)	101 (91-111)
6	16	96 (91-102)	100 (94-106)	101 (93-107)	95(88-103)	86 (80-94)	113 (105-119)	101 (91-111)
7	12	95 (90-101)	97 (91-104)	117 (109-123)	91(84-99)	104 (97-111)	108(100-115)	82 (75-94)
Group mean		**95 (90-101)**	**99 (93-105)**	**104 (97-111)**	**97(90-105)**	**98 (91-105)**	**101 (95-109)**	**91 (82-101)**

FSIQ: Full Scale Intelligence Index (based on the total combined performance of the WAIS-IV subindexes VCI: Verbal Comprehension Index; PRI: Perceptual Reasoning Index; WMI: Working Memory Index; PS Processing Speed Index. GAI: General Ability Index (based on the six subtests that the VCI and PRI comprise). MI: Memory Index (based on the total combined performance of Rey Auditory Verbal Learnin Test, Logical Memory I and II from Wechsler Memory Scale-III and Rey Osterrieth Complex Figure recall

The MRI did not show any pathological changes consistent with previous hypoxemia in any patient. However, in four patients there were other abnormalities: one patient had a small pituitary, one patient had an arachnoid cyst, and two patients had small old cerebral or cerebellar infarctions, and in one of these patients the lesions were known before the ECMO episode.

IV

Due to major problems with sampling and chemical analyses, we were only able to get a full set of data in six patients (two children, one adolescent and three adults). Patient characteristics are presented in Table 17.

Table 17. Patient characteristics before ECMO and outcome

Patient	Age (years)	Sex	Diagnosis	P/F ratio (mmHg)	ECMO days	Survival to DC
1	3	F	ALL, PCP	56	21	yes
2	15	F	Pneumococcal pneumonia	73	7	yes
3	72	M	Streptococcal group A Pneumonia	57	19	yes
4	24	F	Influenza A, Staph. aureus (PVL)	50	15	yes
5	9	M	Influenza	39	9	yes
6	62	M	Influenza	59	55	no

ALL: acute lymphatic leukemia; PVL: Panton-Valentine leukocidin; PCP: Pneumocystis jiroveci pneumonia; P/F ratio: ratio arterial oxygen tension and inspiratory oxygen fraction ($FiO_2 = 1$) before ECMO; DC: discharge from ECMO unit.

All patients had severe ARDS and sepsis. The median duration of ECMO in the surviving five patients was 15 days (IQR = 8–20 days). Blood samples for ECMO early were taken on average 3 days after cannulation for ECMO (IQR = 1–5.5 days), and for ECMO late after 14 days of treatment (IQR = 6.5–18 days) in survivors. In patient #6, who died after 55 days of ECMO, the samples were taken day 40 for ECMO early and day 54 for ECMO late. The first sample was obtained late after cannulation because the patient was transferred to our department 31 days after start of ECMO.

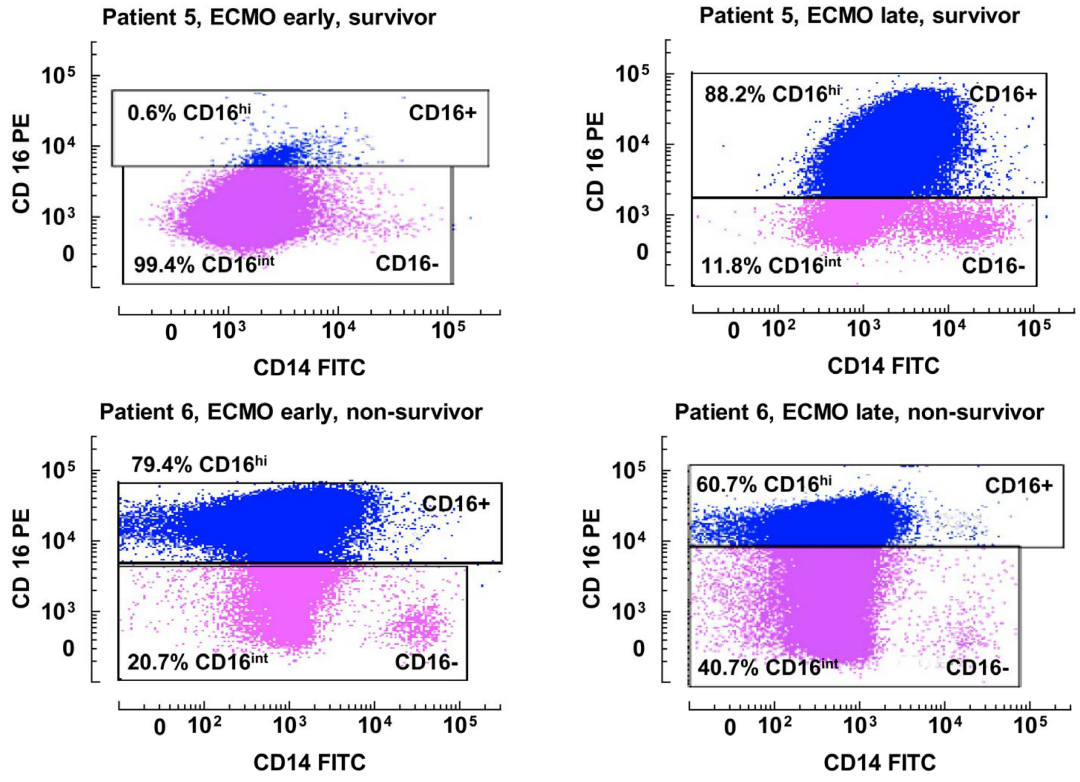
The percentage of $CD16^{hi}$ PMNs from the total CD16 positive cell count in the analyzed sample was lower in the surviving patients compared to the healthy control early after admission to our ICU (48% vs. 99%) and had increased to almost normal levels in the surviving group (90%) at the end of ECMO treatment ($p = 0.03$). The percentage of $CD16^{int}$ PMNs from the total CD16 positive cell count in the analyzed sample were elevated in the survivors early during ECMO compared with the healthy control and decreased significantly during treatment. The differences in CRP, PCT, and WBC counts were not statistically significant (Table 18, Figures 9–11).

Table 18. CD16 polymorphonuclear leukocytes, their C-X-C chemokine receptor 1 (CXCR1) expression, and traditional parameters for the evaluation of the infectious/inflammatory state

Parameter	Healthy control	Survivors early	Survivors late	p-value	Non-Survivor early	Non-Survivor late
WBC x10(9)/L	3.5–8.8	14.2 (8.8)	11.9 (4.9)	0.653	20.5	21.4
CD16 positive cells (count)	-	151 064 (21 573)	125 838 (28393)	0.17	160 713	138 074
$CD16^{hi}$ (%)	99%	48 (30)	90 (5)*	**0.03**	79	61
$CD16^{hi}$ CXCR1 (%)	99%	81 (21)	92 (16)	0,341	98	98
$CD16^{int}$ (%)	1%	53 (30)	10 (5)*	**0.03**	21	41
$CD16^{int}$ CXCR1 (%)	1%	58 (36)	34 (24)	0.174	25	38
CRP mg l^{-1}	< 3	221.2 (145.8)	135 (121.3)	0.172	315	238
PCT µg l^{-1}	< 0.5	9.1 (4.3 - 1093)	1.6 (0.5 - 8.9)	0.063	2.8	1.6

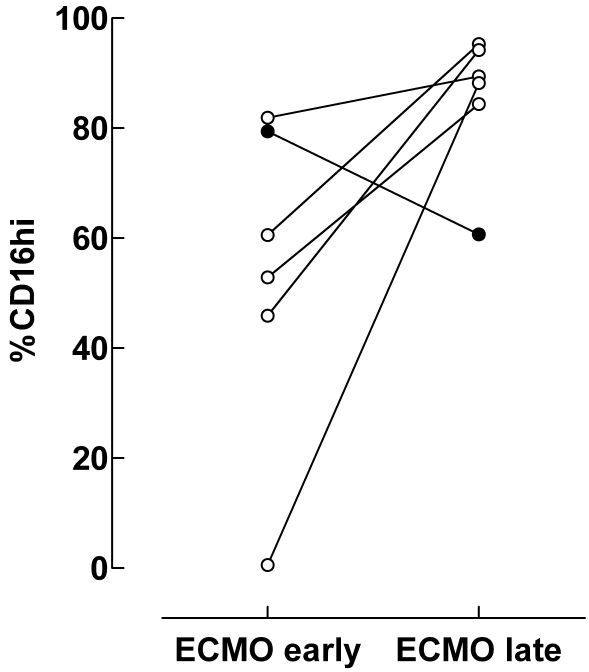
* Indicates a statistically significant difference. Mean ± SD are displayed in parentheses. For PCT the Wilcoxon signed-rank test was performed; the median and interquartile range (IQR) are displayed.

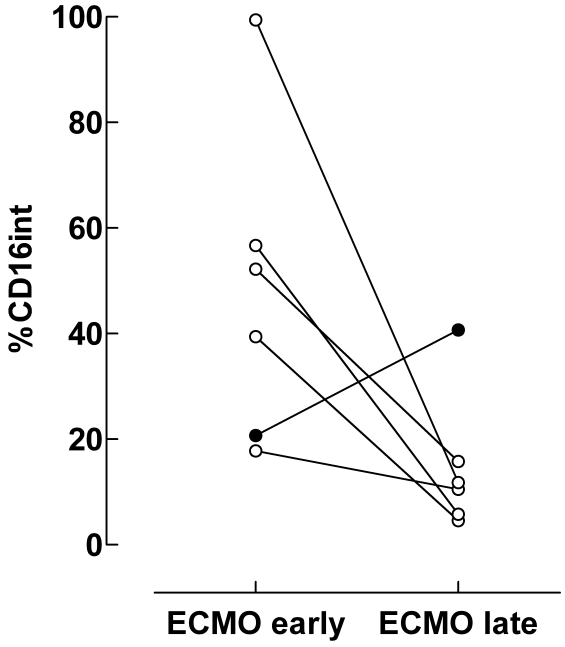
Figure 9. Light scatter plot from fluorescence activated cell sorting of the proportions of $CD16^{int}$ in a survivor (patient #5) and in the non-surviving patient.



The expression of CD16 on PMNs in a survivor (patient 5) and the non-survivor. Mature PMNs express high numbers of CD16 ($CD16^{hi}$) on their surface, while immature PMNs express intermediate numbers of CD16 ($CD16^{int}$). CD16 on PMNs was identified by phycoerythrin (PE)-labeled CD16 antibodies. The PMNs were identified by CD14 antibodies labeled with fluorescein isothiocyanate (FITC).

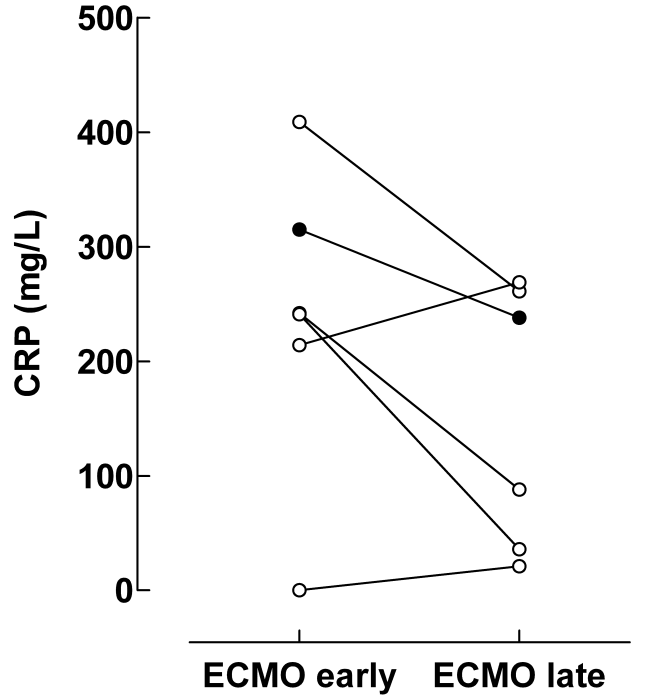
Figure 10. Proportions of mature and immature polymorphonuclear leukocytes (PMNs) in ECMO patients after admission and at the end of treatment.

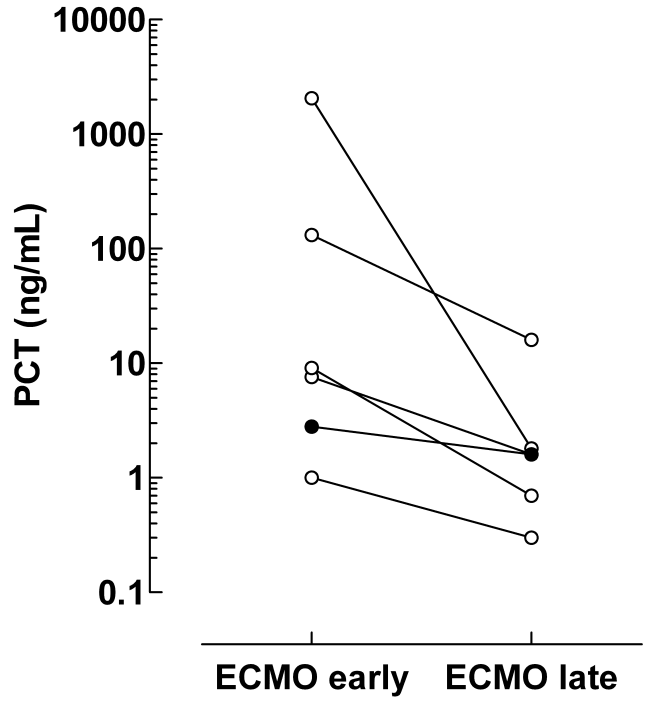


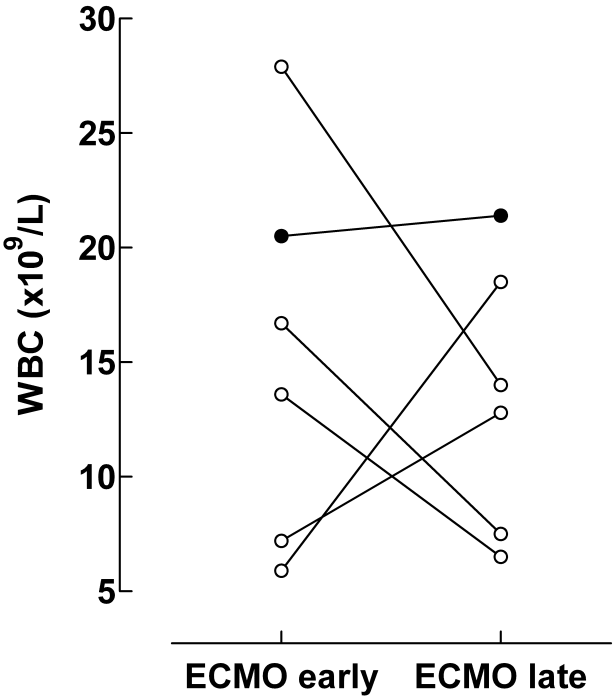


% $CD16^{hi}$: proportions of mature PMNs of CD16 positive cells. $\%CD16^{int}$: proportions of immature PMNs of CD16-positive cells. The filled symbols represent the numbers in the non-surviving patient.

Figure 11. Plasma concentrations of plasma C-reactive protein, procalcitonin, and white blood cells







P-CRP: Plasma C-reactive protein; WBC: White blood cell count (x10^9/L); PCT: procalcitonin. The filled symbols represent the numbers from the non-surviving patient.

Results of cytokine and chemokine analysis are shown in Table 19.

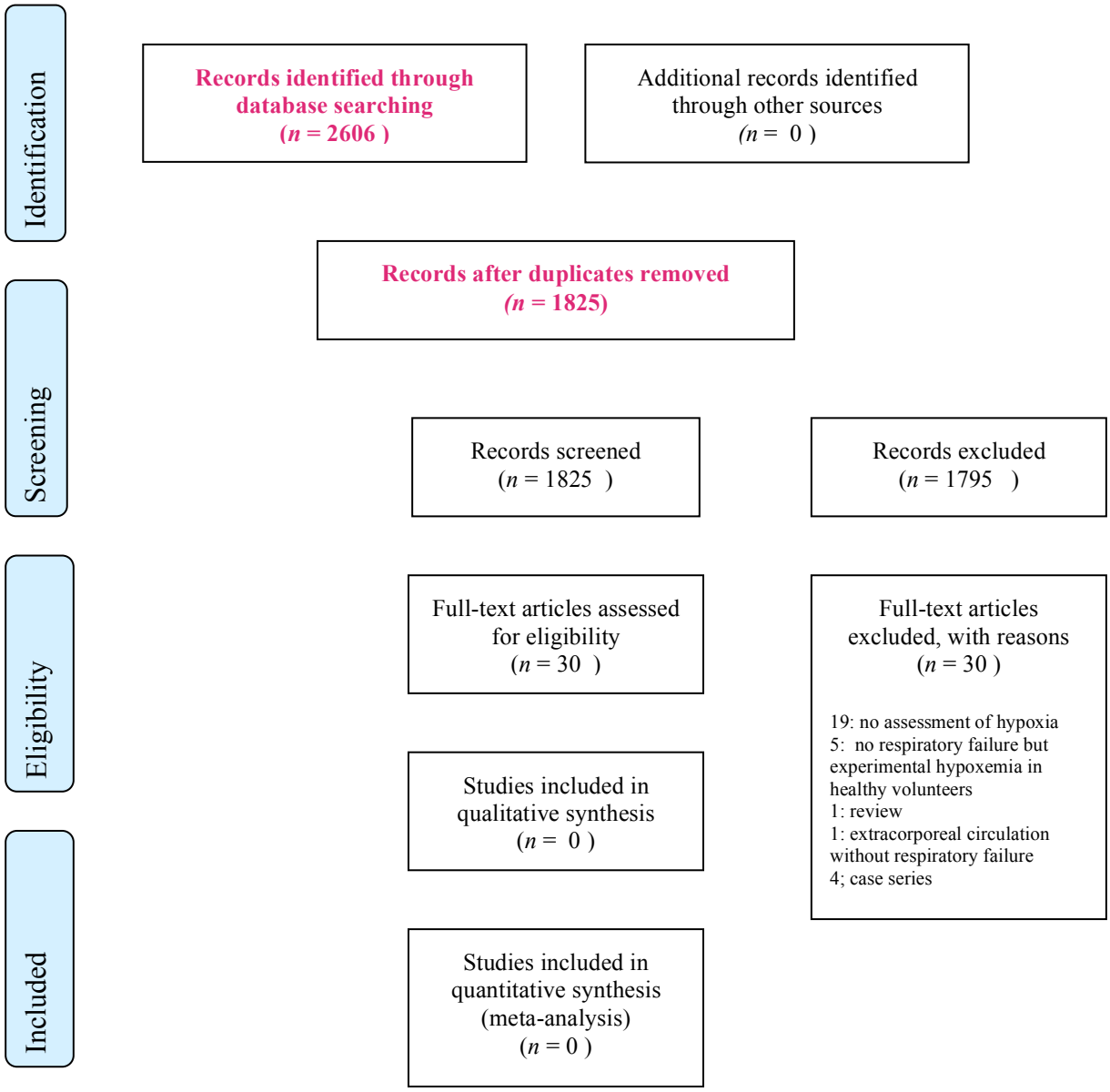
Table 19. Cytokines and chemokines in survivors and one non-survivor

Parameter	Healthy control, mean (*N* = 4)	Survivors early	Survivors late	*p*-value	Non-Survivor early	Non-Survivor late
Eotaxin pg/ml	407	280.6 (282.4)	239.2 (246.6)	0.508	189	449
g-csf pg/ml (IQR)	20.7	10 000 (14 - 10000)	41.3 (13 - 650.9)	0.125	22.1	187
gm-csf pg/ml	3.5	13.6 (14.1)	2.5 (1)	0.136	2.9	8
fraktalkine pg/ml (IQR)	87.5	55.3 (49.1 - 329.6)	60.5 (14.4 - 84.3)	0.313	49.7	85
Ifn alfa pg/ml	18.6	42.4 (56.8)	18.3 (27.1)	0.156	5.1	15
Ifn gamma pg/ml	3.6	7.7 (10.2)	4.8 (7.7)	0.665	0.6	2
Gro pg/ml	1891	2035 (2553.6)	829.3 (474.8)	0.313	207	724
Il 10 pg/ml (IQR)	5.4	105.5 (10.2 - 817.5)	12.9 (5.9 - 63.7)	0.125	28	34
Mcp 3 pg/ml	16.7	266.2 (367.1)	13.7 (10.5)	0.190	28	52
Il12p40 pg/ml (IQR)	19.8	25.4 (9.3 - 70.5)	10.3 (3.2 - 20.9)	0.250	4	42
Mdc pg/ml (IQR)	656	97 (59.3 - 1689.9)	202.5 (49.9 - 455.8)	1.000	200	941
Il 15 pg/ml	1.4	**12.3 (6)**	**3.8 (1.5)***	**0.035**	12	8
Il ra pg/ml (IQR)	14.4	84.2 (31.1 - 2221.9)	12.4 (2.9 - 24.6)	0.063	42	123
sil2 ra pg/ml	21.9	1665.8 (1354.3)	1054.1 (832.3)	0.187	206.8	263.4
Il 1a pg/ml	4.5	14.1 (14.8)	5.1 (3.6)	0.251	2.6	15.7
Il4 pg/ml	6.5	25.4 (16.6)	7.8 (7.9)	0.096	1.6	17
Il 5 pg/ml	0.7	1.2 (0.9)	1.1 (0.5)	0.897	0.8	1
Il 6 pg/ml	2.8	2014.2 (2152.7)	112.1 (121.9)	0.111	30	592
Il 7 pg/ml	2.9	6.4 (7.7)	3.2 (8.1)	0.429	0.8	5
Il 8 pg/ml (IQR)	10.7	96.2 (21.9 - 290.3)	54.6 (19.6 - 90.1)	0.063	74	204
Ip 10 pg/ml	318.1	6891.1 (5149.8)	4147.6 (4329.7)	0.418	2524	10 000
Mcp 1 pg/ml	306	4055.3 (5526)	857.1 (649.8)	0.224	1350	2656
Mip1a pg/ml (IQR)	3.2	9.6 (3.2 - 26.8)	3.2 (3.2 - 3.2)	0.250	3.2	4.4
Mip 1β pg/ml	26.8	**65.3 (39.3)**	**22.9 (11.4)***	**0.031**	20	38
Tnf alfa pg/ml (IQR)	4.2	29.8 (6.4 - 74.4)	8.3 (5.5 - 15.4)	0.063	14	31
Tnf beta pg/ml	3.3	3.3 (2.8)	2.4 (1.3)	0.475	3	3
Vegf pg/ml	78.8	153.6 (158.2)	45.3 (40.1)	0.175	18	76

V

The search was finalized on 26 August 2016. A total of 2606 articles were found. After removal of 781 duplicates, 1825 titles and abstracts were screened. Thirty papers were identified for full text assessment. However, no article met the inclusion criteria. A PRISMA 2009 flow chart was used for reporting items (Figure 12) [45].

Figure 12. PRISMA 2009 Flow Diagram



Six articles reporting case series or describing study questions similar to the one under investigation are described in the review [46-51]. The number of included participants per study ranged from 28 to 102; in total, 270 patients completed the investigations.

Hypoxemia and cognitive dysfunction

Two of the mentioned studies detected an association between decreased blood oxygenation (SaO_2 < 90%) and cognitive impairment, while cognitive impairment occurred in all patient groups treated for ARF/ARDS with either mechanical ventilation or mechanical ventilation and ECMO [46,47]. The percentage of patients with cognitive sequelae ranged from 23% (6 years after discharge) to 100% (at discharge).

In the follow-up study by Hopkins et al., no further change in cognitive functioning was detected between 1 and 2 years after discharge (45% versus 47% cognitive sequelae, respectively) [46]. One study found an association between cognitive dysfunction and a PaO_2 of 71 mmHg compared to a higher PaO_2 of 86 mmHg [51]. Table 20 and 21 show the chracteristics, variable definitions and results of the analysed studies.

Table 20. Characteristics of the studies

Author, year of publication	Study design	Control group	Main exposure	Study size	Mean age, years	Follow up time	Respiratory failure/ARDS stage	Exclusion
Hopkins RO, 2004	Prospective longitudinal outcome study	None	ARDS patients from a ventilation trial; duration SaO_2 <90%	evaluated: n = 78 completed dc n = 74 completed 1 year follow-up: n = 66	45.8 (16-81)	1year	All/P/F ratio ≤150 mmHg	Premorbid cognitive disability, traumatic brain injury, neurologic disease, psychotic disorder
Rothenhäusler H-B, 2001	Case series	Normative population mean	ARDS: n = 40 ECMO: N = 6	evaluated: n = 119 included: n = 46	41.5 (+/- 14.7)	Median 6 years (range 1-12 years)	All/unclear	none
Risnes I, 2006	Case series	None	n = 28, v-v: n = 12 v-a: n = 16	n = 28	37.9 (18.8 - 63.5)	Mean 5 years (0.5 – 12 years)	11/unclear	age <18 years
Hopkins RO, 1999	Case series	Normative population mean	Duration of desaturation with SaO2 <90% <85% and <80%	enrolled: n = 106 study completed at 1 year follow up: n=55	45.5 (16 – 78)	Dc and 1 year	All/unclear	Irreversible disease states, 1 year survival unlikely, enrolled in another study, immunosuppression, CNS damage, inability to obtain informed consent, severe ARDS > 21 days, malignancy, primary care physician refused consent, chronic renal failure, pregnancy, pneumonectomy, chronic heart failure
Hopkins RO, 2005	Longitudinal outcome	Premorbid IQ estimation with OPIE of the study participants	Duration of desaturation SaO_2<90%	evaluated: n = 120, included: n = 74	46 (35-57)	2 years	All/unclear	2 with cognitive disability, 1with Alzheimer's disease
Mikkelsen ME, 2012	Prospective case series	Normative population mean	Hypoxaemia, no definition	consented: n = 213 tested: n = 102 completed all domains : n = 75	49 (40-58)	12 month	All/unclear	None

ECMO: extracorporeal membrane oxygenation; ARDS: acute respiratory distress syndrome; P/F ratio: ratio of arterial oxygen partial pressure to fractional inspired oxygen; v-a ECMO: veno-arterial ECMO; v-v ECMO: veno-venous ECMO; dc: discharge; SaO_2: peripheral measured hemoglobin oxygen saturation; OPIE: Oklahoma Premorbid Intelligence Estimation method;

Table 21. Variable definition and results of the described investigations

Author	Definition of hypoxaemia	Mean time of hypoxaemia (hours)	Definition of cognitive impairment	Number and percentage of patients with cognitive impairment	Correlation of hypoxemia and cognitive impairment	Correlation of ECMO and cognitive impairment	Statistics
Hopkins RO, 2004	SaO_2 <90%	SaO_2 105.9 hours ± 127.6 hours <90%	Two or more tests > 1.5 SD below normative population mean or 1 test below 2 SD below	46 of 66 at dc (69.9%) 30 of 66 at one year (45%)	At discharge? Not significant at 1 year, data not shown	-	McNemar
Rothenhäusler H-B, 2001	not defined	not defined	SKT score of IQ	11 of 46 (23.9%)	not tested	Not significant, p=0.330 numbers not shown	Mann-Whitney U test, Wilcoxon signed rank test, Kruskal- Wallis test, Spearman correlation
Risnes I, 2006	not defined	not defined	1 SD below normative population mean	16 of 28 (57%). 8 of 16 with v-a ECMO (50%) 4 of 12 with v-v ECMO (33%)	not tested	Not significant, numbers not shown	Wilcoxon signed rank test
Hopkins RO, 1999	SaO_2 <85%	<90% = 122 ± 144 hours <85% = 13 ± 28 hours <80% = 1 ± 3 hours	Comparison with corrected t-scores from normative population	55 at dc (100%) 30% 17 of 55 after 1 year in WAIS-R (30%) 43 of 55 in WMS-R and RAVLT (78%)	PaO2 at enrollment was significantly related to cognitive outcome FSIQ impairment for SaO2<90%, p=0.00, SaO2 <85%, p=0.008, SaO2<80%, p=0.013	-	ANOVA, paired t-test and Pearson correlations
Hopkins RO, 2005	SaO_2<90%	<90% = 106 ± 128 hour	2 or more test deviation greater than 1.5 SD or greater 2SD in one test	Patients who completed 1 year follow up: 46 of 66 at dc (70%) 30 of 66 at 1 year (46%) Patients who completed 2 year follow up: 29 of 62 (47%)	Not significant at 1 and 2 years of follow up, no p value shown	-	Descriptive, RMANOVA, Pearson correlation
Mikkelsen ME, 2012	Not defined	unclear	Hayling Sentence Completion Test Score	41 of 75 (55%)	Lower PaO_2 was significantly associated with cognitive impairment at 12 months (p=0.02). PaO2 was no longer significantly associated in secondary analysis (p=0.32)	-	Multivariable logistic regression

BGA: blood gas analysis; SaO_2: hemoglobin oxygen saturation; IQ: intelligence quotient; v-a ECMO: veno-arterial ECMO; v-v ECMO: veno-venous ECMO; dc: discharge; SKT: short cognitive performance test for assessing memory and attention; WAIS-R: Wechsler Adult Intelligence Scale-Revised; WMS-R: Wechsler Memory Scale-Revised; RAVLT: Rey Auditory Verbal Learning Test; FSIQ: Full Scale Intelligence Quotient.

ECMO and cognitive dysfunction

Risnes et al. reported the possible correlation between ECMO treatment and cognitive dysfunction in patients treated with ECMO due to ARF or other conditions [48]. Twelve of 28 studied patients were treated with veno-venous ECMO (v-v ECMO), 11 of these patients because of respiratory failure. Patients treated with veno-arterial ECMO (v-a ECMO) had a higher prevalence of cerebral lesions than patients treated with v-v ECMO (75% and 7% cerebral lesions, respectively, p = 0.0004). Their results showed no direct association between ECMO treatment and cerebral dysfunction, but cerebral lesions possibly due to extracorporeal circulation (CPB or ECMO) were significantly correlated to the degree of cognitive impairment (p = 0.03). Of the patients treated with v-a ECMO, 50% showed neuropsychological impairment compared with 33% in the v-v ECMO-treated patients [48].

Rothenhäusler et al. did not find any indication in their analysis of six cases that ECMO was correlated with cognitive impairment when using ECMO as a risk factor [49].

Study quality

The grade of evidence in the described investigations was low, because there were no direct control groups in any of the studies and no blinded assessment of cognitive impairment. Application of the list of study design features confirmed that the described investigations were before-and-after studies or case series. Thus, the Cochrane list of bias and the Newcastle-Ottawa scale tables were not applicable.

One report had cognitive impairment at study start as an exclusion criterion [50]. The other investigations did not explicitly have a measure to exclude cognitive impairment at study start. The cognitive impairment as outcome was not blindly assessed.

However, the follow-up time was longer than 6 months in all investigations and the drop out lower than 20% in five of six studies.

5 DISCUSSION

The articles presented in this thesis showed that ECMO is a feasible treatment option in patients with pandemic -influenza A / H1N1 2009-induced refractory severe ARDS with a good short- and long-term survival (papers I and III). The Italian EMOnet developed a preECMO morbidity score that showed that the mortality in ECMO-treated H1N1 patients is associated with preECMO extrapulmonary organ function. In this study, the patients from paper I were part of an external validation group (paper II). Seven of 11 survivors were followed up with an extensive test battery to study their cognitive function 3 years after ECMO. The intelligence quotient (FSIQ) and memory function (MI) were all normal despite prolonged hypoxemia during ECMO (paper III). In a systematic review of the current literature, no evidence could be found for the hypothesis that hypoxemia and/or ECMO per se have an impact on cognitive outcome in survivors from severe respiratory failure (paper V). Patients with severe respiratory failure and sepsis are in a hyperinflammatory condition, and paper IV showed in a prospective, observational pilot study that

the proportions of mature and immature polymorphonuclear leukocytes differ significantly between the early and late phase of ECMO treatment.

Paper I

Thirteen patients with confirmed pandemic influenza A/H1N1-induced severe ARDS were treated with ECMO at the ECMO Department of the Karolinska University Hospital, Solna, Sweden. All patients survived to discharge from ECMO treatment, and 12 patients were still alive three months after discharge from hospital (92%). This study is the largest single center study on patients treated with ECMO for H1N1 respiratory failure. Other single or multicenter studies have reported survival rates between 35% and 79% [52, 53]. Several factors may have contributed to the results presented in paper I: a) we cannulated and started ECMO before and continued during the transport from the referring hospital as conventional transportation to an ECMO center is associated with substantial mortality; b) we used a sufficient oxygen supply by ECMO to meet the oxygen requirements of the patients, making it possible to establish low airway pressure, low tidal volume ventilation and even accepting totally atelectatic lungs and discontinuation of ventilation in case in which pneumothorax occurred; c) we changed the circuit from veno-venous to veno-arterial ECMO when right heart failure occurred; and d) needed major surgery was performed, although reoperation due to postoperative bleeding was required in one case.

Paper II

PreECMO hospital length of stay; creatinine, bilirubin and hematocrit values and mean arterial blood pressure were significantly associated with mortality in patients treated with vv ECMO for influenza A H1N1 2009. Based on these finding, the ECMOnet score was developed. This score had a high accuracy for the prediction of the mortality risk in the patients treated with ECMO (ROC analysis: c = 0.857, 95% CI, 0.754–0.959, $p < 0.001$). The probability of correctly classifying patients with this score was 75%, where a score of 4.5 was the most appropriate cutoff for mortality risk prediction. In the external validation group, the score also had a good capacity to distinguish survivors from non-survivors (ROC analysis: c = 0.694, 95% CI, 0.562–0.826, $p = 0.004$). Five of 13 patients from paper I had a score of more than 4.5, i.e., the estimated mortality was > 80 % (Table 22). All these patients survived ECMO treatment.

Table 22. The ECMOnet score of 13 patients treated at the ECMO Centre Karolinska who were part of the external validation group

N	Score	Missing values	ECMO survival
1	6	-	yes
2	4	-	yes
3	3	-	yes
4	2	3	yes
5	1	3	yes
6	8	1	yes
7	4	-	yes
8	1	3	yes
9	1	3	yes
10	1	3	yes
11	6	-	yes
12	8	1	yes
13	6	-	yes

Furthermore, this score was validated in the context of ECMO-treated patients, a relatively homogenous group of patients with severe respiratory failure due infection with H1N1. Whether the ECMOnet score has the same accuracy in other patient populations has not been proven and needs to be investigated. Therefore, the use of this score for decision making for initiating ECMO treatment should be taken with great caution.

Paper III

This is the first study reporting the long-term neurological outcome of permissive hypoxemia in ECMO-treated patients and, furthermore, it is the first reporting the long-term neurological outcome in patients treated with ECMO during the 2009/2010 H1N1 pandemic. We found that cognitive function, including memory, was within two standard deviations of the mean FSIQ in a healthy reference population despite all patients being hypoxemic before ECMO and subjected to permissive hypoxemia during the first 10 days of their treatment or the entire treatment, respectively.

All patients had normal preoxygenator venous saturations and blood lactate values, which indicated normal tissue perfusion, i.e., no tissue hypoxia.

Other studies found a statistically significant correlation between cognitive dysfunction and hypoxemia, i.e. a SaO2 < 90%, but it is not clear whether hypoxemia was the underlying cause of these sequelae [46, 47].

Previous laboratory studies have shown that CaO_2 and not PaO_2 is the most important factor for cerebral oxygenation and perfusion [54]. In our study, mean CaO_2 was kept at 14.3 ± 1.9 ml/100ml by increasing hemoglobin concentration by erythrocyte transfusions. One potential drawback with this approach is the risk of transfusion-related complications. However, in a recent study in septic patients without hypoxemia comparing two transfusion policies resulting in two levels of hemoglobin concentrations, there were no differences in complications between the groups, and in another study in patients with cardiac surgery, likewise without hypoxemia, the mortality rate even decreased with higher hemoglobin level and thus a higher number of transfusions [55, 56]. Moreover, a low preECMO hematocrit value (35% versus 30%, $p = 0.01$), i.e., a low hemoglobin value, was significantly associated with ECMO mortality, as shown in paper II of this thesis.

Paper IV

In this pilot study in six ECMO-treated patients with severe ARDS and sepsis, we observed a statistically significant difference in the proportions of $CD16^{hi}$ and $CD16^{int}$ from ECMO early to ECMO late in the surviving patients. In contrast, we could not find any significant changes in traditionally used markers of inflammation, CRP, PCT, and WBCs. Although we did not assess the inflammatory role of the PMNs in either the lungs or in the blood, our findings of high levels of GM-CSF combined with a high proportion of $CD16^{int}$ in the early phase of ECMO confirm the important role of $CD16^{int}$ in the first phase of ARDS [9]. As expected, the expression of the receptor CXCR1 was considerably increased on these cells. This receptor has a high affinity to the chemokine IL-8, which plays a pivotal role in the chemoattraction and activation of the PMNs [57, 58]. Thus, the combination of increased levels of CXCR1 on $CD16^{int}$ and the IL-8 at the beginning of the ECMO treatment underlines the role of immature PMNs in the inflammatory response. The simultaneously increased anti-inflammatory cytokines interleukin 10 (IL-10) and interleukin 1 receptor antagonist (IL-1ra) could be seen a counter-response to this pro-inflammatory activity [59, 60]. In contrast, in the non-surviving patient $CD16^{int}$, PMNs and anti-inflammatory cytokines were higher in the late than they were in the early ECMO phase. One could speculate whether this could be due to an inadequate anti-inflammatory activity in a situation with increased inflammation. In the surviving patients, we found that the cytokine IL-15 and chemokine Mip 1β decreased during ECMO treatment. Although these results were incidental findings that could have been due to chance, they are in accordance with the notion that the survivors' immune responses were appropriate. Mip 1β is a chemokine, which is produced by macrophages during endotoxin stimulation. It activates PMNs and therefore has a pro-inflammatory effect [61]. The IL-15 is secreted by phagocytes and regulates the activation of natural killer cells, which are necessary for the immune response [62].

The course of the proportions of immature PMNs occurring in the peripheral blood in patients with severe inflammatory conditions like severe ARDS or sepsis treated with ECMO has not previously been investigated. The present study suggests that it may not only have a diagnostic but also a

prognostic value in these patients, but this study has several limitations. First, the sample size is small, and although the study was prospective, we were only able to get full data sets from a fraction of the patients eligible for the study. Therefore we cannot be certain that the patients included are fully representative of the ECMO patient population. Consequently, general conclusions are difficult to draw, and the findings should be interpreted with great caution. Second, the pro-inflammatory effect of the ECMO treatment itself has not been studied in this context. Extracorporeal circulation induces the activation of PMNs and increases cytokine concentrations, which could have affected our findings [63].

Paper V

This review could not identify any RCT, nested case-control study, or longitudinal observational study, i.e., studies with high quality evidence that investigated the relationship between hypoxemia in ventilated patients with ARF and/or ECMO treatment and cognitive outcome. We found six reports with low evidence grades that investigated some of our study questions. In these, the proportion of patients with cognitive dysfunction ranged from 70% to 100% at discharge (Hopkins 1999 and 2004/2005) to 23% to 57% in patients with ARF. The follow-up was 1 to 12 years after discharge.

Two studies investigated the correlation between hypoxemia and cognitive function by assessing the duration of hypoxemia and correlating it statistically with cognition [46, 47, 48]. Two studies reported an association between hypoxemia (SaO_2 < 90%) during treatment and cognitive dysfunction in survivors from severe ARF/ARDS, one at discharge and one at 12 months after discharge [46, 47]. In the study by Mikkelsen et al. mean SaO_2 levels were similar, 94.2% in the groups with "higher" (86 mmHg) and with "lower" (71 mmHg) PaO_2. In fact, this SaO_2 level is not commonly considered as hypoxemic. Moreover, in a secondary analysis, Mikkelsen et al. could not verify their previously found association [51]. The two remaining studies by Rothenhäusler et al. and Risnes et al. did not assess the degree of hypoxemia but dealt with patients treated with ECMO for severe ARF [47, 48]. The study by Risnes et al. in survivors of ECMO treatment for ARF and severe circulatory failure showed that patients treated with v-v ECMO for ARF had less cognitive dysfunction compared with patients treated with v-a ECMO. Indeed, the patients treated with v-v ECMO had similar rates of cognitive dysfunction as reported in non-ECMO-treated patients, namely between 40% and 50% at 1 year after discharge from hospital [48]. Rothenhäusler et al. did not find that ECMO treatment was correlated with cognitive dysfunction [49].

Limitations

In this thesis, I have focused on the treatment of H1N1-infected patients with severe respiratory failure treated with extracorporeal membrane oxygenation. The problem with my approach to study such patients in a single center setting is the low number of patients that can be included. Thus, my studies, except study number V, can be considered to be case series. Consequently, the results I have been able to obtain can only give an indication that our treatment policy is acceptable and as good as that in other ECMO centers. However, I believe that it is ethically questionable to perform a randomized controlled study to evaluate whether the outcome from ECMO treatment versus conventional ventilation in patients referred to our internationally

recognized ECMO unit due to two reasons; 1) the conventional treatment used before initiating ECMO by definition has failed, and it is highly probable that the patients will not survive without ECMO. In this context it is important to recognize that the patients are incompetent to give informed consent by themselves due to sedation and the disease process. And 2) the number of patients treated in our unit is too small to get adequate statistical power to conclusively decide whether ECMO is associated with improved outcome regarding survival and cognitive function. Unfortunately, the few randomized studies regarding ECMO have not been conclusive, and no studies as we found out in the systematic review regarding hypoxemia/ECMO treatment have been able to pinpoint whether hypoxemia or ECMO treatment are associated with long-term cognitive dysfunction. Thus, in my view, the limited evidence I provide in my studies is as good as that presented in the current literature. Nevertheless, my studies should be interpreted cautiously due to these limitations.

6 CONCLUSIONS

- Patients treated with ECMO for H1N1 induced acute respiratory failure have a good short- and long-term outcome in terms of survival and cognitive functioning. To achieve positive results with this treatment option, it is necessary to provide an ECMO transportation service that allows initiating ECMO at the referring hospital and thereby stabilizing the patient before and during conventional transport and possibly reducing transportation-associated mortality risk. Other factors possibly influencing the outcome are the change from v-v ECMO to v-a ECMO in case of right ventricular incompensation, performing major surgery when necessary, and treatment of these patients in specialized ECMO departments with sufficient case loads.
- Survival of ECMO patients is related to preECMO extrapulmonary organ function and the ECMOnet score may be helpful in estimating the mortality risk in ECMO patients before initiating ECMO. Using the score to deny ECMO to patients fulfilling the criteria because their mortality risk would be too high does not seem to be appropriate, and as the score only has been studied in H1N1 patients, it needs to be confirmed in larger studies with a more heterogeneous patient population.
- Despite a severe hypoxemic condition before and during ECMO, the follow-up study of seven ECMO-treated patients with influenza A/H1N1 infection had normal cogntive function 3.2 years after ECMO. These results indicate that the ELSO recommendation of a SaO2 down to 80% during ECMO may be reasonable with respect to long-term cognitive outcome if this level allows preservation of adequate organ perfusion. Because of the small sample size, further studies in this category of patients are needed.
- In our prospective, observational pilot study, normalization of the proportions of $CD16^{hi}$ and $CD16^{int}$ in patients treated with ECMO for refractory ARDS with sepsis was associated with an improvement of the clinical situation in survivors. Thus this time course of these proportion could be used as a prognostic marker in these patients. The predictive value of the

clinical routine measurements of CRP, PCT, and WBCs was limited in the patients in this pilot study. Due to the small sample size, our study is only hypothesis generating. This observation needs therefore to be evaluated in studies with larger sample sizes.

- Our systematic review did not identify any high quality study answering the question whether hypoxemia during conventional mechanical ventilation or ECMO treatment, or ECMO itself, is associated with long-term cognitive impairment. However, from low evidence data we cannot exclude that hypoxemia could influence cognitive dysfunction at discharge from hospital in patients treated with only mechanical ventilation. We consider that it is necessary to examine the question whether hypoxemia and interventions such as ECMO treatment have effects on short- and long-term cognitive outcome in future RCTs. In addition, further evidence is required to determine the effect of other factors that could affect cognition, especially the modifiable factors, like metabolic state, oxygen transport, and organ perfusion pressures.

7 ACKNOWLEDGEMENTS

I want to express my sincere gratitude to my supervisor Professor Ander Larsson who is the most professional person I have ever met. He always gave me support when needed.

My dear children,
Please apologize that this work took so much of my free time from you. You never complained but I know that there were enough occasions. Thank you for your love and patience.

Håkans friendship is invaluable.

Leif, Gunilla, Marita, Rita and the whole ECMO staff,
Thank you so much. Without your help and hard work the studies I, III and IV could not have been finalized.

I am very grateful to my co-supervisors Professor Björn Frenckner and Associate Professor Peter Radell for their help during the studies, the half-time board and with editing and writing the manuscripts.

Study IV was a cooperation with the Department of Medicine, Translational Immunology Unit of the Karolinska University Hopsital and Karolinska Institutet. I want to thank Professor Ola Winqvist, Petra Jones and all the others who were involved for their help and their support.

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